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Influence of soil moisture on the incidence and severity of leaf spot (*Alternaria alternata*) in *Glycine max* genotypes

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ABSTRACT

Background/Objective. The soybean crop is affected by various diseases caused by fungi, which can severely reduce yields, in some cases up to 100%. The objective of this study was to identify the fungus associated with leaf spot in soybean and to evaluate the influence of soil moisture on the incidence and severity of the disease in plant and leaf in 30 soybean genotypes.

Materials and Methods. Three soybean leaves with leaf spot symptoms were collected per genotype for morphological identification of the fungus using taxonomic keys. Two moisture conditions (irrigated and rainfed) were evaluated. Plant incidence was estimated from 10 randomly selected plants; during the same period, foliar incidence and severity were evaluated. For foliar incidence, healthy and diseased leaves were counted from 10 plants per genotype. Subsequently, from 10 plants per genotype, one leaf in the fifth position from top to bottom was selected. The leaves were photographed, and the damaged area and total leaf area were calculated using the ImageJ software, thereby determining leaf severity.

Results. *Alternaria alternata* was identified as associated with leaf spot. In the rainfed environment, leaf incidence by *A. alternata* ranged from 4.4 to 50.55%; for severity, the values ranged between 0.3 and 17.4%. In the irrigated environment, the incidence varied from 7 to 50.9%; the severity ranged from 0 to 24.7%.

Conclusion. *A. alternata* was associated with leaf spot in soybean, which is little studied in Mexico, and currently its effects on this crop are unknown; therefore, it is of scientific interest to continue working on research projects with this species.

Keywords: Disease, Leaf blight, Leaf spot, *Glycine max*

INTRODUCTION

The soybean crop (*Glycine max*) is one of the most important grains worldwide, is widely used in the food industry for both humans and animals, as well as in biofuels (Pratap *et al*., 2012; Cabanos *et al*., 2021; Zhang *et al.,* 2022). The seed contains between 40 and 42% of protein, and it has been estimated that this legume is the source of 25% of the edible oil manufactured worldwide and two-thirds of the protein concentrate used for cattle food (Agarwal *et al*., 2013; Liu *et al*., 2020; Zhao *et al*., 2022). On a global scale, the countries with the largest production levels are Brazil, United States, Argentina, China and India, which jointly represent approximately 85% of the world's production (Guo *et al*., 2022; Gupta and Manjaya, 2022). In Mexico, this oleaginous plant is grown in Campeche, Veracruz, Yucatán, Chiapas, Tamaulipas, San Luis Potosí, Quintana Roo, Sonora and Sinaloa, with a total production in 2024 of 230 863.19 tons in a surface of 124 951.33 ha (SIAP, 2024).

The main threats to the production of soybean are climate change, edaphic factors such as pH and electrical conductivity, as well as weeds, insects, viruses and diseases (Bandara *et al*., 2020; Pozebon *et al*., 2020; Lin *et al*., 2022). Regarding diseases, the soybean crops is mentioned to be susceptible to root and leaf diseases, as well as those that affect the seedling stage. These diseases can be caused by diverse microorganisms such as the soybean cyst nematode (*Heterodera glycines*), bacteria (*Pseudomonas savastanoi*), and fungi that cause charcoal rot (*Macrophomina phaseolina*), soybean downy mildew (*Peronospora manshurica*), soybean target spot (*Corynespora cassiicola*), soybean stem canker (*Diaporthe aspalathi*), leaf blight or purple seed stain (*Cercospora kikuchii*), sudden death syndrome (*Fusarium* sp.), Asian rust (*Phakopsora pachyrhizi*), soybean white mold (*Sclerotinia sclerotiorum*) and leaf spot (*Alternaria alternata*) (Reznikov *et al*., 2019; Roth *et al*., 2020; Bradley *et al*., 2021; Fagodiya *et al*., 2022). These microorganisms can lead to significant losses in yields of 11 to 50%. In the United States alone, between 1996 and 2016, approximately 23 diseases were observed in the soybean crops, in the 28 producing states, where economic losses of 95 480 million dollars were observed (Bandara *et al*., 2020).

The fungus *A. alternata* causes the disease known as leaf spot or leaf blight in several agricultural crops. In soybean, this disease is one of the most destructive in leaves, and the climate plays an important part in the pathogenicity and in the processes of infection and spreading (Fagodiya *et al*., 2022; Buzdar *et al*., 2023). In 2018, in Sinaloa, in northern Mexico, symptoms of leaf spot were observed in the grain-filling stage and the onset of maturity in soybean plants of the Nainari variety; from the fungal isolates, the *Curvularia* species was identified and associated with the leaf spot symptoms (González-Molotla *et al*., 2021). In the period of 2020 and 2021, in the Heilongjiang province, China, a rare leaf spot was observed in soybean, from which the pathogen was identified as *A. alternata*, making this the first report on the disease in northeastern China (Li *et al*., 2023). In India, in the region of Maharashtra, leaf spot caused by *Alternaria* spp. was reported as an important disease in the soybean crop. According to reports, the incidence of this disease fluctuated between 12.5 and 41.6% (Zade *et al*., 2018). In turn, Fagodiya *et al*. (2022), mention that *A. alternata* causes leaf spot, and it is considered a destructive disease in soybean crops in Rajasthan, India, where a leaf damage rate of 58.2% is mentioned in the RKS-24 cultivar.

In Mexico, although leaf spot has already been reported as a disease in the soybean crop, it was associated to *Curvularia* sp., and therefore, *A. alternata* is a little-known phytopathogenic fungus in Mexico, which can be found in association with leaf spot in soybean. To date its effect and the damages it can cause in the main production states is unknown. Therefore, the aim of this study was, the aim of this study was to identify the causal agent that induces leaf spot in 30 soybean genotypes and to evaluate the influence of soil moisture on the incidence and severity of this disease on plant and leaf.

EXPERIMENTAL DEVELOPMENT

Location and establishment of the experiment. The evaluation was conducted at Las Huastecas (CEHUAS) Experimental Field, belonging to the National Forestry, Agriculture and Livestock Research Institute (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias - INIFAP), located in the municipality of Altamira, Tamaulipas, in geographic coordinates 22º 33' 57.88'' north latitude and 98º 09' 52.47'' west longitude, at an altitude of 17 m a.s.l. The experiment was established in the spring-summer cycle of 2022 in a late plantation (August 23, 2022) of 30 soybean genotypes. The identification of the fungus and the evaluation of the incidence and severity of the disease was carried out by observing a natural leaf spot infestation in the field. Two moisture conditions were evaluated (irrigation and rainfed). In the irrigated treatment, four irrigation depths were applied using a drip irrigation system: pre-sowing (13 mm), at the time of sowing (13 mm) and 12 (19 mm) and 45 (26 mm) days after sowing (DSS) in addition to the rainfall accumulated during the growing cycle (426.6 mm). For the rainfed treatment, the plants were grown under rainfed conditions. Only a 13 mm pre-sowing irrigation was applied, along with the accumulated rainfall. Sowing was carried out manually by direct seeding. The soybean genotypes were planted in plots consisting of two rows each with a row spacing of 0.76 m and a length of 5 m, resulting in a usable plot area of 7.6 m^2, and a density of 20 plants per linear meter.

Identification of the causal agent. In October, in the S-S cycle of 2022, soybean leaflet were found with chlorosis and later necrosis in the reproductive stage between R5-R6. Three leaf and root samples were taken for each genotype evaluated, and the samples were stored in an ice cooler during sampling to avoid dehydration; they were later transported to the Laboratory of Toxicology of the Las Huastecas Experimental Field. In the lab, 4 cm^2 squares were cut from leaves with symptoms, along with 1 cm pieces of roots, which were disinfected in ethanol at 75% for 5 s and in NaOCl at 2% for 2 min, followed by rinsing with sterilized distilled water for 3 min. The plant material was dried with sterile absorbent paper, then placed in moist chambers, which consisted of 12 × 12 cm Petri dishes containing moist sterilized paper, kept at room temperature (≈25-28 °C), with a relative humidity of 70 to 80% (Jiang *et al.,* 2018). Fungal identification was carried out with the taxonomic keys by Simmons (2007) and Rodríguez-Roa *et al.* (2013); for this purpose, the width, length, number of transverse septa and number of longitudinal septa were taken from 300 conidia.

Evaluation of the incidence on the plant. On the 99th DAS, the visual incidence of the disease per plant per genotype was evaluated. For this, 10 plants (5 per furrow) located within the 3 central meters of the two furrows for each soybean genotype, were chosen. A careful review for the presence or absence of the symptoms of the disease was carried out (irregular yellow leaf spots /irregular dark brown necrosis with yellow edges). A plant was considered diseased when chlorosis without necrosis was found on the leaf of a plant. The incidence on plants was estimated using the following formula: Incidence on plant (%) = (Diseased plants/ Total plants) x 100 (Herrera and Laurentin, 2012).

Incidence and severity on the leaf. For leaf incidence, healthy and diseased leaves were counted on 10 plants, and this was used to calculate the percentage of incidence on leaves using the following formula: Incidence on leaf (%) = (Diseased leaves / Total leaves) x 100 (Felipe-Victoriano *et al*., 2025). For severity, the fifth leaf from the top down from 10 random plants per genotype were chosen, cut and placed on a white 30*30 cm panel, where they were photographed with a SONY DSC-H300 camera, helping determine the damaged area and the total area of the leaf using the ImageJ program. Subsequently, leaf severity was estimated using the formula: Severity = (Damaged leaf area / Total leaf area) x 100 (Rasband, 2018). A one-way ANOVA was performed, and the comparison of means was carried out using Duncan's test (α=0.05) on the INFOSTAT statistical software (2023).

Identification of fungi. From the root samples, only in one did *Sclerotinia sclerotiorum* develop, a phytopathogenic fungus with a broad host range, among which are the roots and stems of the soybean crop (Zhu et al., 2022). Whereas in leaflets under temperature conditions of ≈25 to 28 °C and 80% relative humidity, the development of conidiophores was observed from which conidia developed, which allowed the identification of *A. alternata* as a species associated with leaf spot in soybean. From the foliar lesions, the only fungus that was observed was *A. alternata*.

The fungus *A. alternata* in the soybean crop was found in the field as irregular yellow leaf spots, marked strongly from the onset of the infection (Figure 1A). Subsequently, where the chlorosis begins, an irregular dark brown necrosis with yellow edges develops, which can cover a large part of the leaf (Figure 1A). In the soybean folioles, the concentric rings, typical of the *Alternaria*, cannot be perfectly noticed; when visible, they are quite dull. In the moist chambers, conidiophores and chains of conidia were observed (Figure 1B). At 40X, straight, dark brown conidiophores were observed 40X above the foliole. The conidia measured between 23.3 and 57.2 μm in length by 8.0 – 13.2 μm in width, with 2 to 5 transversal septa and between 0 and 3 longitudinal ones (Figure 1C).

Figure 1. *Alternaria alternata* on soybean, **A**) Leaves with symptoms, **B**) Signs observed under the stereoscopic microscope at 4X, **C**) *A. alternata* conidia observed under the light microscope at 40X.

In 2019, in Turkey and Pakistan, similar symptoms to those described in this study of the soybean were observed. In the isolation, *A. alternata* was identified, presenting dark conidia with one or two transversal septa and between four and nine longitudinal septa. The dimensions of the conidia were 29 to 59 × 13 to 26 μm, similar morphometric characteristics to those found in this study (Ustun *et al*., 2019; Buzdar *et al*., 2023).

Regarding this, in Argentina, Larran *et al*. (2002), mention that *A. alternata* is an endophytic phytopathogenic fungus in the soybean crop, in which signs and symptoms were observed, beginning on stage R2. Subsequently, in the south and east of Rajasthan, India, Fagodiya *et al*. (2022) reported *A. alternata* as one of the most destructive fungal diseases for the soybean crop.

The incidence of the leaf spot induced by *A. alternata* in plants depended on soil moisture. The highest number of plants with symptoms and signs of *A. alternata* were found in the treatment in the irrigation environment (Figure 2). Under rainfed conditions, genotypes H10-3057, H02-1706, H98-1076, Huasteca 600, Huasteca 700 and H17-0882 presented an incidence per plant between 30 and 60%, whereas in the irrigation environment, genotypes H10-3057, H98-1076, Huasteca 600 and Huasteca 700 presented an incidence between 50 and 60% (Figure 2).

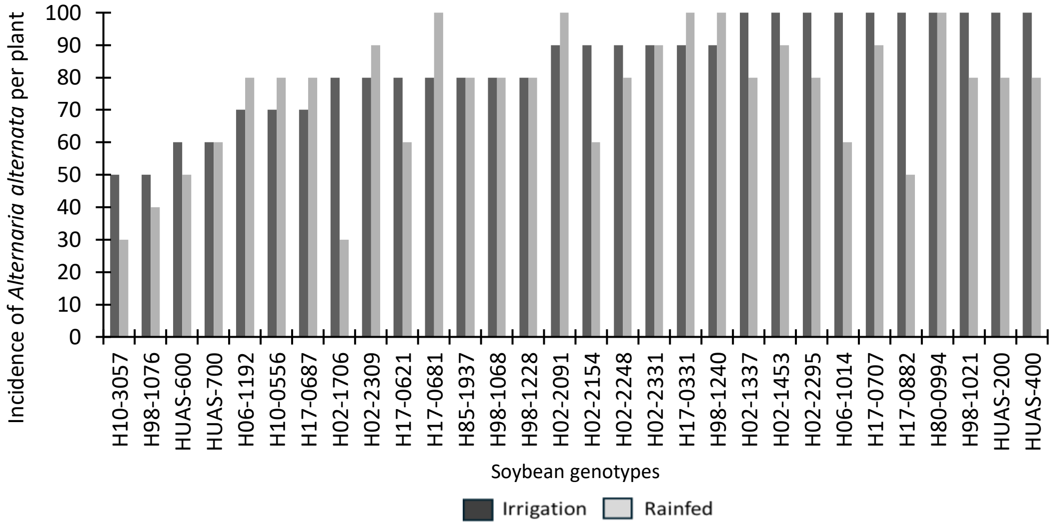

Figure 2. Incidence of leaf spot per plant caused by *Alternaria alternata*, in soybean genotypes under conditions of irrigation and rainfed in Altamira, Tamaulipas, 2022. HUAS = Huasteca.

In the irrigation treatment, 33.3% of the genotypes presented a 100% incidence, whereas in the rainfed treatment, only 16.6% presented this percentage of incidence (Figure 2). In this regard, Zade *et al*. (2018) mentioned that in the region of Vidarbha, in India, *A. alternata*, the cause of leaf spot in soybean, presented an incidence between 12.5 and 41.6%. This low incidence in the disease could be due to this region presenting a semi-arid climate area, where temperatures can reach up to 48 °C in the summer, and these conditions do not favor the development of fungal diseases, as in the case of regions with a subhumid warm climate, such as in southern Tamaulipas, Mexico, where an incidence of leaf spot in plants can range from 30 to 100%. Likewise, Zade *et al*. (2018) mention that in fungal isolations, other *Alternaria* species can be found, such as *A. tenuissima*, which had an incidence of less than 3.8% (Zade *et al*., 2018).

The incidence and severity of leaf spot (*A. alternata*) under rainfed conditions presented significant statistical differences between treatments (p-value <0.0001). In the rainfed treatment, the disease appeared in 4.4 and 50.5% of the leaves of the evaluated genotypes. Genotypes H10-3057, H02-1706, H17-0621, H98-1076, H02-2154 presented the lowest foliar incidence of *A. alternata*, which ≤ 10% of leaves with symptoms of the disease. Regarding the severity caused by *A. alternata*, values fluctuated between 0.3 and

17.4%. Out of all the materials, 66.6% presented damage below 8.4%, highlighting genotypes H02-1706 and HUAS-700, which displayed a greater tolerance to *A. alternata*, since their foliar damage was ≤ 0.8% (Table 1 and Figure 3).

Table 1. Incidence and severity on the leaf of leaf spot caused by *Alternaria alternata*, on soybean genotypes under irrigation and rainfed conditions in Altamira, Tamaulipas, 2022.

Rainfed conditions					Irrigation conditions				
Soybean genotypes	Incidence (%)		Severity (%)		Soybean genotypes	Incidence (%)		Severity (%)	
H10-3057	4.4 (± 2.4+)	a[z]	1.5 (±1.0)	ab	H98-1076	7.0 (± 2.9+)	a[z]	7.3 (±4.3)	abc
H02-1706	5.6 (± 3.3)	ab	0.3 (±0.3)	a	HUAS-700	7.6 (± 2.4)	a	6.2 (±2.7)	abc
H17-0621	6.1 (± 1.9)	ab	2.6 (±2.6)	abc	H10-3057	13.1 (± 5.5)	abc	0.0 (±0)	a
H98-1076	7.8 (± 3.3)	abc	1.3 (±0.6)	ab	HUAS-600	17.2 (± 6.0)	abc	5.9 (±4.9)	abc
H02-2154	10.0 (± 8.3)	abcde	13.6 (±5.9)	cd	H17-0621	21.7 (± 6.5)	abcde	3.8 (±2.9)	ab
HUAS-700	10.1(± 4.0)	abcd	0.8 (±0.5)	ab	H02-1706	23.4 (± 6.2)	abcde	0.0 (±0)	a
H06-1014	10.8 (± 4.2)	abcd	15.5 (±4.1)	d	H98-1068	24.7 (± 5.5)	abcdef	11.5 (±4.7)	abc
H85-1937	11.9 (± 2.9)	abcd	1.4 (±1.4)	ab	H17-0687	25.5 (± 7.5)	abcdefg	4.5 (±1.6)	abc
HUAS-600	13.9 (± 6.1)	abcde	1.9 (±0.8)	abc	H98-1021	26.8 (± 6.8)	abcdefgh	6.9 (±3.1)	abc
H17-0882	14.6 (± 6.0)	abcde	6.3 (±2.7)	abcd	H17-0331	27.2 (± 4.8)	abcdefgh	17.8 (±6.7)	cd
H17-0687	16.0 (± 4.6)	abcde	2.4 (±2.2)	abc	H85-1937	27.8 (± 6.3)	abcdefgh	1.6 (±0.8)	a
HUAS-400	18.6 (± 5.6)	abcde	8.4 (±4.1)	abcd	H98-1228	29.2 (± 7.4)	abcdefgh	6.3 (±3.5)	abc
H98-1228	20.5 (± 5.6)	abcdef	2.1 (±1.5)	abc	H10-0556	31.1 (± 9.4)	bcdefgh	7.6 (±3.1)	abc
HUAS-200	23.6 (± 5.6)	abcdefg	6.6 (±2.8)	abcd	H02-2154	33.8 (± 5.9)	cdefgh	7.8 (±3.3)	abc
H06-1192	24.1 (± 4.7)	abcdefg	6.4 (±3.9)	abcd	H06-1192	34.7 (± 11.0)	cdefgh	10.0 (±4.3)	abc
H02-2248	25.3 (± 7.1)	bcdefgh	12.9 (±5.9)	bcd	H06-1014	36.0 (± 4.5)	cdefgh	3.51 (±2.2)	ab
H02-1337	27.4 (± 7.2)	cdefghi	7.4 (±3.3)	abcd	H17-0681	37.9 (± 10.8)	defgh	10.5 (±3.5)	abc
H98-1021	27.5 (± 7.3)	cdefghi	7.1 (±3.7)	abcd	H02-2331	38.3 (± 5.2)	defgh	10.7 (±4.9)	abc
H02-1453	28.1 (± 8.8)	cdefghi	14.5 (±4.3)	d	H98-1240	41.3 (± 7.3)	efgh	9.4 (±4.1)	abc
H10-0556	29.0 (± 8.5)	defghi	1.5 (±1.1)	ab	H02-2309	41.3 (±8.9)	efgh	8.2 (±3.6)	abc
H98-1068	30.4 (± 6.9)	defghi	2.3 (±0.9)	abc	H02-1337	41.4 (± 4.0)	efgh	4.9 (±2.5)	abc
H17-0707	32.5 (± 4.2)	efghij	14.7 (±4.0)	d	H02-2295	42.0 (± 8.0)	efgh	6.2 (±3.0)	abc
H98-1240	39.4 (± 4.3)	fghij	2.4 (±1.2)	abc	H02-2248	42.3 (± 9.5)	efgh	6.1 (±2.6)	abc
H02-2295	40.5 (± 9.3)	fghij	16.7 (±4.5)	d	HUAS-400	47.9 (± 8.7)	fgh	5.6(±2.7)	abc
H80-0994	41.2 (± 4.7)	ghij	12.9 (±6.1)	bcd	H02-1453	48.1 (± 5.7)	fgh	7.7 (±4.2)	abc
H02-2091	41.6 (± 6.6)	ghij	12.0 (±5.6)	abcd	H17-0707	48.5 (±6.7)	fgh	10.8 (±3.2)	abc
H02-2331	42.9 (± 7.4)	ghij	17.4 (±4.8)	d	H02-2091	49.4 (± 9.9)	gh	15.6 (±6.4)	bcd
H17-0681	44.3 (± 5.2)	hij	14.5 (±5.4)	d	H17-0882	50.0 (± 7.2)	gh	5.7 (±2.6)	abc
H02-2309	45.5 (±10.2)	ij	6.4 (±2.5)	abcd	HUAS-200	50.6 (± 5.3)	h	7.5 (±5.2)	abc
H17-0331	50.5 (± 4.9)	j	6.6 (±1.9)	abcd	H80-0994	50.9 (± 4.2)	h	24.7 (±6.7)	d
p-value	<0.0001		<0.0001		p-value	<0.0001		0.0143	

[z]Different letters indicate different statistics between treatments (Duncan; α = 0.05), +standard deviation. HUAS = Huasteca.

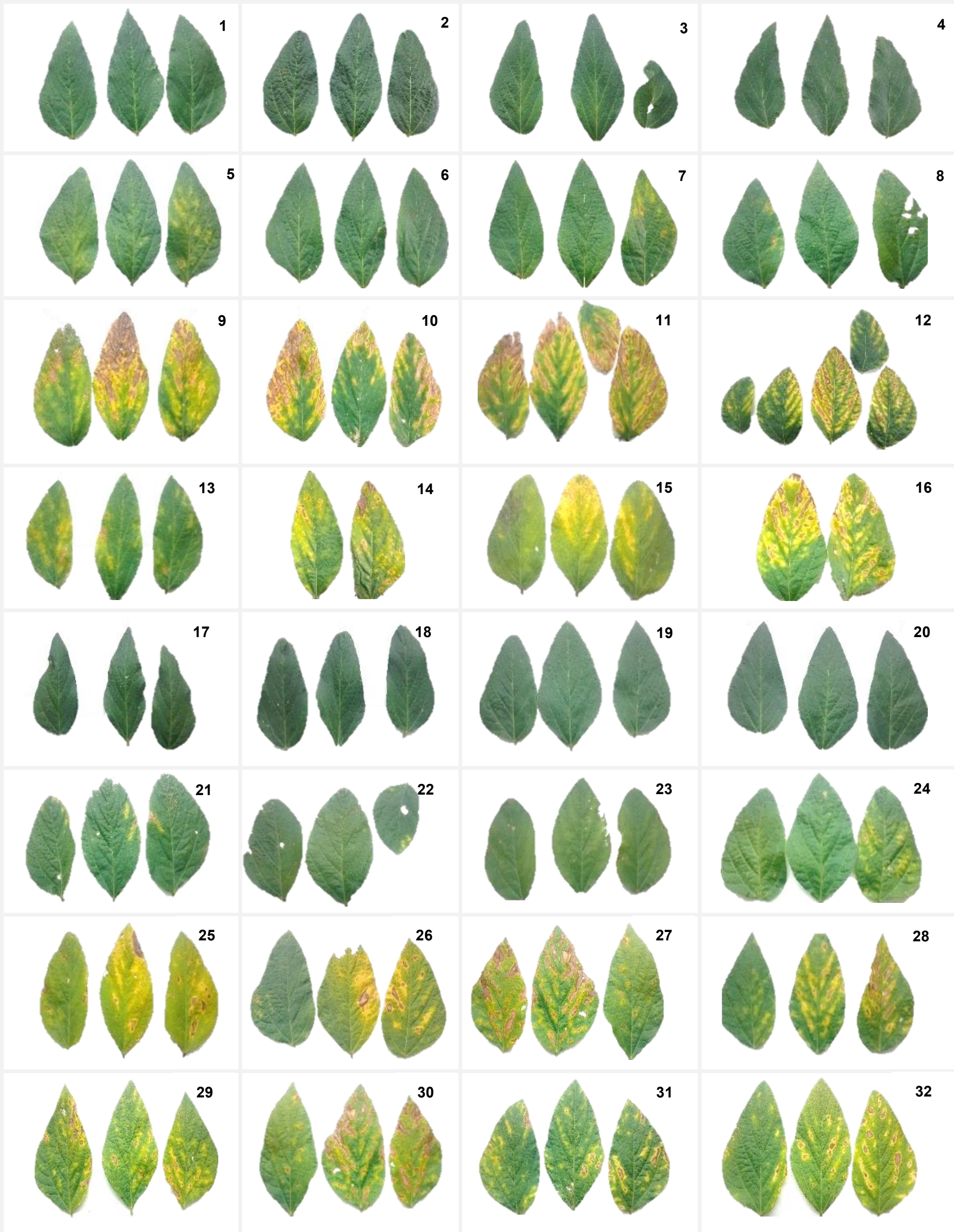

Figure 3. Symptoms of leaf spot in soybean, induced by *Alternaria alternata*, leaves located in the fifth position from top to bottom in irrigated soybean genotypes, (genotypes with less damages) H10-3057 (**1-2**), H02-1706 (**3-4**), H85-1937 (**5-6**), H06-1014 (**7-8**); (genotypes with more damage) H80-0994 (**9-10**), H17-0331 (**11-12**), H02-2091 (**13-14**), H98-1068 (**15-16**); rainfed conditions, (genotypes with less damage) H02-1706 (**17-18**), HUAS-700 (**19-20**), H98-1076 (**21-22**), H85-1937 (**23-24**); (genotypes with more damage), H02-2331 (**25-26**), H02-2295 (**27-28**), H06-1014 (**29-30**), H17-0707 (**31-32**).

The incidence and severity of *A. alternata* leaves under conditions of irrigation presented significant statistical differences between treatments (p-value <0.0001 and 0.0143). Table 1 shows that, under conditions of irrigation, a plant can present between 7 and 50.9% of its leaves with symptoms of leaf spot associated with *A. alternata*. For this humidity condition, only two genotypes (H98-1076 and Huasteca 700) displayed an incidence of the disease in their leaves of less than 10%. Regarding leaf severity caused by

A. alternata, values ranged from 0 to 24.7%. It is important to highlight that, under irrigation conditions, 76.6% of materials presented a severity ≤ 10% (Table 1, Figure 3).

The leaf spot caused by *A. alternata* in soybean is one of the emergent diseases in the crop in the last 8 years, which can present an incidence (12 to 41.6%) and severity (58.2%), due mostly to climate change (Fagodiya *et al.,* 2022). In Mexico, it is a little-known disease in the soybean crop. Its effects on the crop are currently unknown; the literature reports that it is more prone to developing epiphytotics in crops located in humid and warm-humid areas. This corresponds to observations in this study, which found that *A. alternata* presented a higher percentage of incidence in the treatments with higher irrigation levels in comparison with rainfed treatments. Fagodiya *et al.* (2022) mention that another important factor for the development of epiphytotics by *A. alternata* is the temperature; they point out that temperatures between 15 and 34 °C are adequate for the development of the disease.

Conclusions

According to macro and micrometric morphological characteristics (conidiophores and conidia), *A. alternata* was identified as associated with leaf spot in the soybean crop. On plants and leaves, the incidence of *A. alternata* depended on the humidity in the soil: the higher the humidity, the higher the incidence and severity became in the different genotypes evaluated. Genotypes H10-3057, H98-1076, Huasteca 600 and Huasteca 700 presented the lowest incidence (< 17.2) and severity values (< 7.3) in both environments, which would indicate that they probably present a certain degree of tolerance to leaf spot in this crop.

Conflict of interest. The authors declare not to have any conflict of interest in this manuscript.

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