



Perspective

Combating aflatoxin contamination by combining biocontrol application and adapted maize germplasm in northeastern and southeastern Mexico

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HIGHLIGHTS

- AF36 Prevail® biocontrol reduced aflatoxin contamination in maize fields by 59.0% to 89.9% in northeastern and southeastern Mexico over four years.
- Treated fields had less ear rot, with some maize hybrids showing higher yields and aflatoxin levels below 20 ppb.
- Biocontrol combined with adapted maize germplasm improves safety and productivity, especially in climate-affected regions.
- The study supports AF36 Prevail® registration in Mexico and promotes research on native atoxigenic strains for better biocontrol.

ARTICLE INFO

Keywords:

Zea mays L
Mycotoxins
AFB1
Aspergillus flavus
AF36-Prevail®

ABSTRACT

Maize is highly vulnerable to aflatoxin (AF) contamination caused by fungi from the *Aspergillus* section *Flavi*, with deficiencies in post-harvest management practices further exacerbating AF levels. Due to their carcinogenic properties, AFs pose significant health risks. Biological control using non-aflatoxigenic *A. flavus* isolates has been effective for over 25 years in the USA, with two formulations being commercially available. However, no such products have been developed yet for use in Mexico. This study evaluated the effectiveness of AF36-Prevail®, a non-aflatoxigenic strain from Arizona, for reducing aflatoxin contamination in Mexico. Over four years (2019–2022), we assessed its impact alongside regionally adapted maize germplasm in northeastern and southeastern Mexico. We analyzed a total of 1,479 grain samples, with 887 from biocontrol-treated fields, and 592 from untreated fields across 69 sites in Tamaulipas and Campeche. Treated fields showed 59.0% to 89.9% reductions in AF content compared to untreated fields, and higher ear rot was observed in untreated fields. Correlation coefficients between ear rot and AF content were $r = 0.08$ for Campeche and $r = 0.36$ for Tamaulipas. Significant differences ($p \leq 0.001$) were noted between years and hybrids for both yields and AF levels. Three hybrids in Tamaulipas and four in Campeche demonstrated better adaptation, higher yields, and lower AF levels (< 20 ppb). This research underscores the potential for safer maize production in Mexico, particularly when combining biocontrol strain application with adapted germplasm.

1. Introduction

Aflatoxins (AFs) are highly toxic secondary metabolites primarily produced by specific strains of the fungus *Aspergillus flavus*, *A. parasiticus*, and *A. nomiae*, with *A. flavus* being the most common (Xing et al., 2017). Among the four major aflatoxins (B1, B2, G1, G2), aflatoxin B1 (AFB1) holds significant concern due to its carcinogenic properties in both animals and humans, and it is frequently found in various foodstuffs worldwide (Mahato et al., 2019). Chronic exposure to AFB1 by

frequent ingestion of foods with low doses is associated with severe health issues, including hepatocellular carcinoma, reduced immunity, growth impairment, and non-alcoholic cirrhosis, particularly in malnourished children (Lewis et al., 2005). Conversely, acute exposure by ingestion of grains and/or feeds contaminated with high AFB1 concentrations primarily affects livestock and poultry, but it can also occur in humans. This leads to symptoms like vomiting, abdominal pain, pulmonary edema, liver failure, and necrosis, with a higher mortality rate compared to chronic exposure (Dhanasekaran et al., 2011; Richard

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<https://doi.org/10.1016/j.biocontrol.2025.105727>

Received 13 September 2024; Received in revised form 10 February 2025; Accepted 15 February 2025

Available online 27 February 2025

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et al., 2006; Yin et al., 2018).

The effects of climate change on temperature, rainfall patterns, and humidity, along with changes in agricultural practices, post-harvest management, and the natural alterations of fungal populations, including rapid adaptation to field conditions, have led to new patterns of secondary metabolite production, increasing the risk associated with mycotoxins, particularly AF (Miller, 2023; Singh et al., 2023). In terms of maize ear rot, rising temperatures and drought conditions have triggered a geographical transition from *Fusarium graminearum* to *F. verticillioides*, and subsequently from *F. verticillioides* to *A. flavus*. This shift has resulted in a corresponding transition from mycotoxins like deoxynivalenol (DON) and zearalenone (ZEA) to fumonisins (FUM), and from FUM to AF, respectively (Savary et al., 2011).

In Mexico, the presence of AFs in maize grains has been documented in Tamaulipas and Campeche since the 1990 s (Moreno and Gil, 1991; Rodríguez-Del Bosque et al., 1995). AFs have also been detected in Nuevo Leon, Sonora, Sinaloa, Guanajuato, and Chiapas, as well as in food products intended for both human and animal consumption (Espinosa et al., 1995; Méndez-Albores et al., 2004; Sharma and Márquez, 2001). The reports on AF have been limited, primarily focusing on the incidence of *A. flavus* in maize grains and scarce surveillance of AF in food products 2). This situation adversely impacts consumer health, farmer income, and results in significant short-term losses in agricultural and livestock production (Díaz-Franco and Montes-García, 2008; Montes et al., 2009; Padrón et al., 2013). In recent years, the issue has escalated due to climatic factors (high temperature and drought) that favor the occurrence of toxigenic fungi in both field and storage settings (Xu et al., 2022). Most recently, there have been reports on the incidence of AFB1 in serum samples, biopsies, and risk exposures analyses (Díaz de León-Martínez et al., 2020; Lino-Silva et al., 2022; Monge et al., 2023).

Aflatoxin mitigation presents a multi-dimensional challenge that requires actions throughout the entire crop production cycle, encompassing crop management and post-harvest practices, such as grain sorting, drying, storage, and subsequent processing (Odjo et al., 2022). Given that chemical control is not an option and genetically resistant maize varieties against toxigenic strains of *A. flavus* have shown limited efficacy at the field level, the use of biological control through the application of non-aflatoxigenic strains of *A. flavus* is currently the most effective, efficient, economical, ecological, and safe technology to reduce AF contamination in the field (Collinge et al., 2022; Dorner, 2010; Ortega-Beltran et al., 2019). In this sense, several biocontrol formulations have been developed as commercial products, including: AF36Prevail®, whose active ingredient AF36 was isolated from cottonseed in Arizona by the USDA-ARS and commercialized by the Arizona Cotton Research and Protection Council (ACRPC); Afla-Guard®, whose active ingredient fungus was isolated from peanut in Georgia by USDA-ARS, and is now owned by SYNGENTA®; Aflasafe, which is a group of multi-strain formulations for which active ingredient fungi were isolated from maize and groundnut soils by the International Institute of Tropical Agriculture (IITA) in several African countries; and AF-XI®, whose active ingredient fungus was isolated from maize in Italy by the Catholic University of the Sacred Heart, Piacenza and commercialized by CORTEVA (Bandyopadhyay et al., 2016; Bhandari et al., 2020; Cotty, 1997; Dorner, 2010; Mauro et al., 2018). The exact mechanism of biocontrol with *A. flavus* biocontrol strains is unclear, but may occur by biocontrol strains competitively excluding or displacing toxigenic strains present in treated agricultural soils (Cotty, 2006; Sarrocco et al., 2019). There are other potential mechanisms, such as the competition for nutrients (Mehl and Cotty, 2013), thigmoregulation (Huang et al., 2011), and most recently chemosensing through extrolites and volatile organic compounds produced by non-aflatoxigenic isolates (Moore et al., 2022; Sweany et al. 2022).

In Mexico, previous studies on maize soils from Sonora and maize ears from Sonora, Nayarit, Sinaloa, Tamaulipas, and Campeche have shown that members of the vegetative compatibility group (VCG) YV36,

to which AF36 belongs, are also endemic to the region. Members of YV36, including the active ingredient of AF36, predominantly reproduce asexually and are considered genetically stable (Grubisha and Cotty, 2015; Ortega-Beltran, 2012; Ortega-Beltran et al., 2015, 2020; Ortega-Beltran et al., 2016; Ortega-Beltran and Cotty, 2018). Although recombination between *A. flavus* biocontrol strains and native toxigenic strains in the field is possible (Horn et al., 2016), no such events have been reported in Mexico to the best of our knowledge. Therefore, in theory, the technology could be used in Mexico as a result of the NAFTA agreement, this has not been done, and it is currently unknown if AF36 would be effective in Mexico. The aim of the current study was to assess, over four consecutive years (2019–2022), the integrated management approach of applying AF36 biocontrol in conjunction with maize germplasm adapted to specific production areas, for mitigating AF contamination in the states of Tamaulipas (northeastern) and Campeche (southeastern), Mexico.

2. Materials and methods

2.1. Germplasm and description of field sites

The AF36-Prevail® technology and maize germplasm were evaluated from 2019 to 2022 in farmers' fields in the states of Tamaulipas and Campeche, considering farmers' preferences for germplasm, cumulative crop performance data from previous production cycles, land availability, and water resources. The germplasm included commercial hybrids marketed by national and international seed companies. These hybrids were selected based on farmers' preferences, superior productivity, and their availability within the companies' product portfolios. The sequence of hybrid evaluations is described later in the text, as subsequent assessments were determined by their performance in combination with the use of AF36-Prevail®, following a consensus with the farmers. AF36-Prevail® was imported with approval from Mexico's regulatory authority, SENASICA.

2.1.1. Tamaulipas

The state of Tamaulipas, situated in northeastern Mexico, shares its northern border with the USA and its eastern coastline with the Gulf of Mexico. This region, known for its cultivation of maize, sorghum, cotton, and sunflower, predominantly focuses on maize production during December to June/July [fall/winter (FW) season]. Medium-large-scale farmers plant maize and rely on irrigation for their crops. The prevalent soils in this area are xerosols, regosols, lithosols, and fluvisols. The climate is warm, dry, and sub-humid (INEGI, 2023). The period with the highest temperatures spans from May to August, with temperatures averaging 35.5 °C in the daytime and 23.7 °C at nighttime. The lowest temperatures occur in January, with average temperatures of 23.8 °C at daytime and 11.3 °C at nighttime. However, with the arrival of cold fronts from the Gulf of Mexico and the Atlantic Ocean, temperatures can drop close to 0 °C. On an annual average, daytime temperatures are around 31.0 °C, while nighttime temperatures settle at 18.7 °C (CONAGUA, 2023). The highest relative humidity, averaging at 92 %, typically occurs during August and September (Weather-Spark, 2023). Rainfall is concentrated between May and October, resulting in an annual precipitation of 620 mm, without a specific distribution pattern (CONAGUA, 2023). The FW cropping cycle is irrigated, commencing with planting from December to February. Flowering typically initiates approximately 80–100 days later, in March and April, followed by harvests around 150 days later, occurring between June and July (Fig. 1A).

During the FW2018-19 cycle, eight hybrids were assessed in a 10-hectare (ha) area in Empalme, Río Bravo. For the subsequent FW2019-20 cycle, only six hybrids that exhibited low levels of AF in the FW2018-19 cycle were re-evaluated. Moving on to the FW2020-21 cycle, solely the top three hybrids with low AF levels were re-evaluated, in addition to the two hybrids preferred by farmers. The

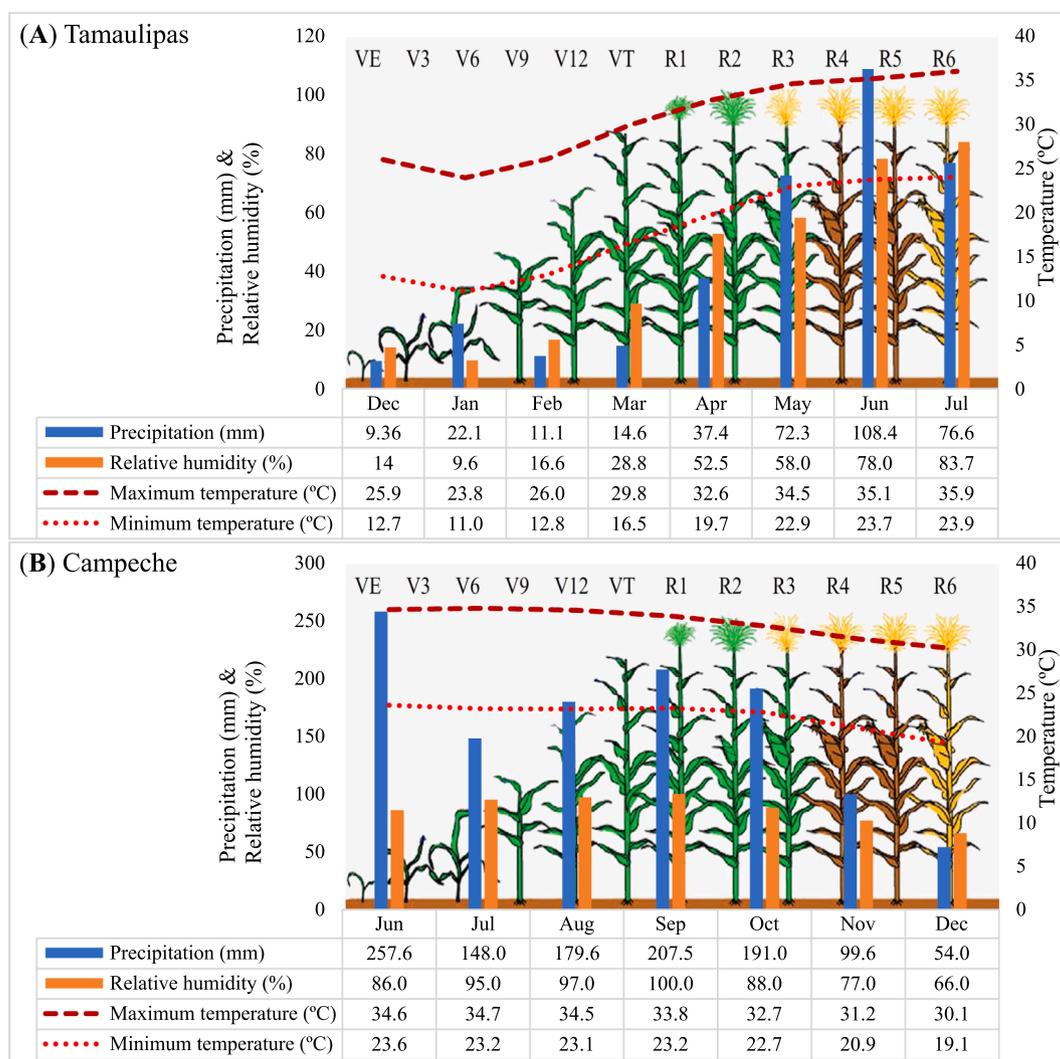


Fig. 1. Average monthly precipitation, relative humidity, and maximum and minimum temperature at corn growth stages during 2019, 2020, 2021 and 2022 in (A) Tamaulipas and (B) Campeche. The applications of the Prevail AF36 product were made from the vegetative stage when the plant has 7 leaves (V7) up to two to three weeks before flowering when the vegetative phase ends (VT) and aflatoxin levels in grain maize sampled at harvest were examined. Note: In Tamaulipas the maturity stage of the crop coincides with high temperature and rainfall, unlike in Campeche which coincides with drought. Both climatic conditions favor the development of the fungus *A. flavus* and aflatoxins when they coincide in the sensitive stage of maize. . Source: CONAGUA, (2023); Weather-Spark, (2023). Picture: PNGitem, (2023)

FW2019-20 and FW2020-21 cycles were planted in eight different sites, comprising 100 ha, spanning three subregions, namely Díaz Ordaz, Río Bravo, and Abasolo, to provide representation for both the northern and central regions of Tamaulipas. In the FW2021-22 cycle, only the three most promising hybrids (AG-2525, NB-722 and P-3057), with the lowest

presence of AF and the highest potential yields, were evaluated across 21 sites, covering a total area of 500 ha (Table 1 and Fig. 2A).

2.1.2. Campeche

The state of Campeche, situated in southeastern Mexico, on the

Table 1

White hybrids were evaluated for aflatoxin mitigation during fall/winter (FW) cycles from 2018 to 19 to 2021–22 in the state of Tamaulipas.

No.	Hybrid	Seed company	2018–19	2019–20	2020–21	2021–22	Characteristics
1	NB-722*	NOVASEM	✓	✓	✓	✓	Excellent stability, and adaptability and tolerance to fusarium
2	AG-2525*	ANZU	✓	✓	✓	✓	Cob health and high yield
3	P-3057*	PIONEER	✓	✓	✓	✓	Precocity, strong stems, and high yield
4	GARAÑÓN**	ASGROW	✓	✓	–	–	Stability and high yield
5	ARMADILLO**	ASGROW	✓	✓	–	–	Excellent initial vigor and cob uniformity
6	HIPOPÓTAMO**	ASGROW	✓	✓	–	–	Excellent vegetative vigor and grain health
7	TGX-8960***	TECH AG	✓	–	–	–	Yield potential
8	TGX-8990***	TECH AG	✓	–	–	–	Yield potential
9	TITAN****	ASPROS	–	–	✓	–	Excellent plant health (Stay Green) and tolerance to fusarium

* Promising hybrids evaluated for four years (2019–2022) with high yield and low incidence of *A. flavus* and aflatoxins.

** Control hybrids used in three evaluation years.

*** Hybrid highly susceptible to *A. flavus* and aflatoxins that was only evaluated in one year.

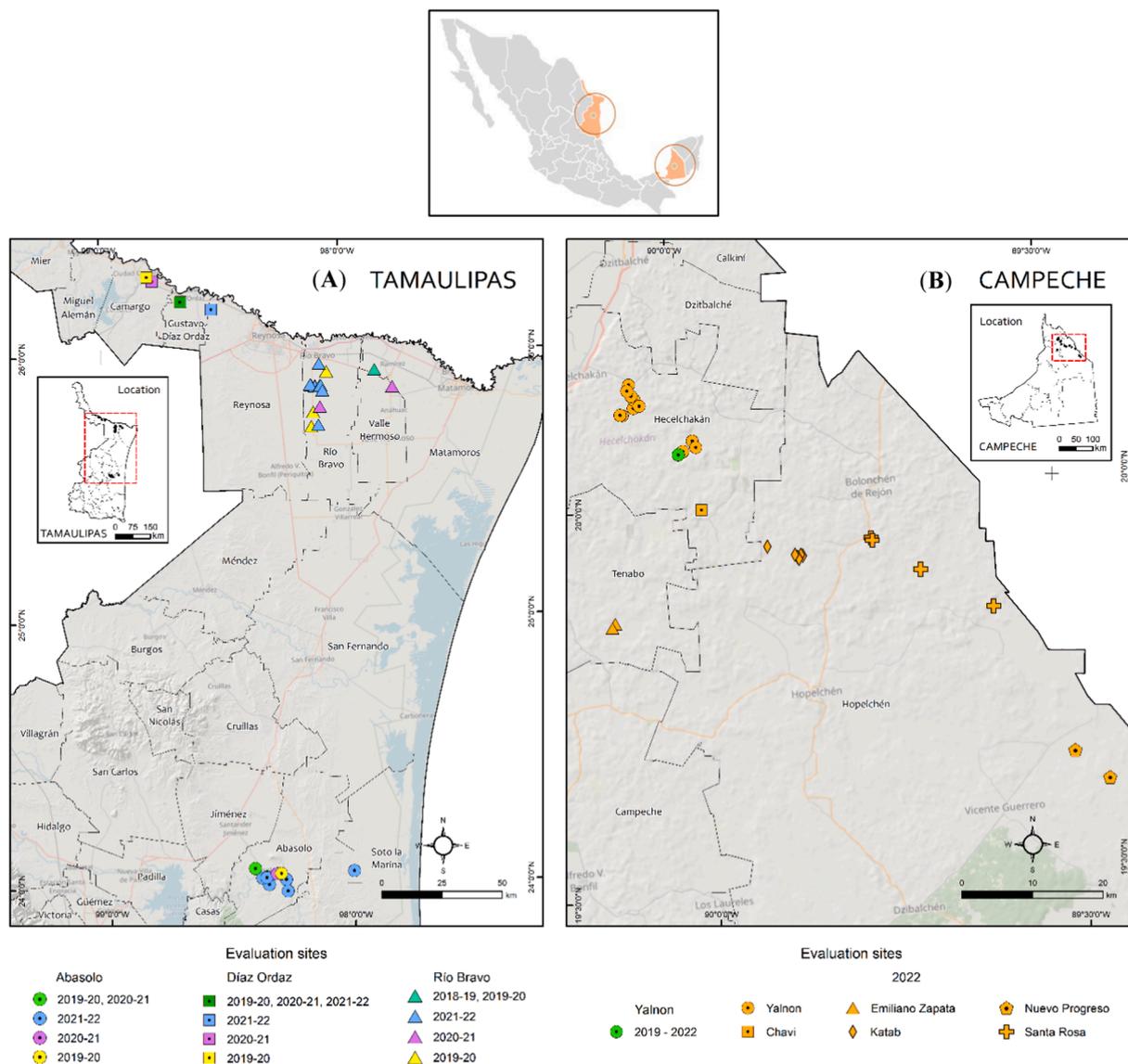


Fig. 2. Evaluation sites for aflatoxin mitigation in (A) Tamaulipas and (B) Campeche during 2019 2020, 2021 and 2022. Note: In Tamaulipas, three sites were established (Abasolo, Díaz Ordaz and Río Bravo) with two and three continuous applications of AF36 product. In Campeche, one site (Yalnon) was established with four annual applications without interruption. These sites served as a reference for the effectiveness of biological control with the atoxigenic strain AF36 Prevail.

western part of the Yucatán Peninsula, shares borders with the Republic of Guatemala to the south and the Gulf of Mexico to the west. The primary focus in Campeche is maize production for local consumption, with a diverse range of producers including small-, medium-, and large-scale operations. The evaluations were carried out in collaboration with medium-scale producers who rely on rainfall for crop development. The lands in Campeche are generally more diverse and smaller in scale compared to those in Tamaulipas. Maize cultivation is specific to the spring/summer (SS) season, from June to November/December. The prevalent soils in this region are reddish (nitrosols) and black (leptosols and gleysols). It has warm and cloudy climate prevailing throughout the year (INEGI, 2023). The highest temperatures are experienced in May, with a daytime temperature of 36.5 °C during the day and 23.5 °C at nighttime. On an annual average, daytime temperatures reach 33.3 °C, while nighttime temperatures settle at 21.7 °C (CONAGUA, 2023). The period from July to September has the highest relative humidity, averaging 97 % (Weather-Spark, 2023). Rainfall is concentrated between June and October, resulting in an annual precipitation of 1,400 mm, without a specific distribution pattern (CONAGUA, 2023). The SS crop cycle is dependent on rainfall, with planting taking place in June-July,

flowering occurring at approximately 55 days, and harvest occurring around 120 days later, in late November (Fig. 1B).

In SS2019 and SS2020, nine hybrids developed for the southern zone of Mexico were evaluated on a 10 ha plot in Yalnon, Hecelchakán. In SS2021, the four hybrids with the lowest incidence of AFs from the previous two years, along with five additional hybrids were assessed in the same field. For SS2022, only four promising hybrids (CORONEL, P-4028, P-4279 and TORNADO) with less *Aspergillus* ear rot, low presence of AFs, and highest yield potential were evaluated over a total area of 512 ha, distributed across 28 sites in six localities in the municipalities of Hecelchakán and Hopolchén (Table 2 and Fig. 2B).

2.2. Application of biocontrol AF36Prevail®

The AF36Prevail® product consists of sterilized sorghum grains, without germination capacity in the field, and coated with conidia of the AF36 strain (NRRL18543) along with a distinctive blue dye to distinguish them from regular sorghum and discourage consumption (Cotty, 1997). The sorghum grains serve as a carbon source for the fungus, providing it with a competitive edge over toxigenic strains in the soil

Table 2

White hybrids were evaluated for aflatoxin mitigation during spring/summer (SS) cycles from 2019 to 2022 in the state of Campeche.

No.	Hybrid	Seed company	2019	2020	2021	2022	Characteristics
1	P-4028*	PIONEER	✓	✓	✓	✓	Good foliar and grain health
2	P-4279*	PIONEER	✓	✓	✓	–	Good foliar and grain health
3	TORNADO*	CERES	✓	✓	✓	–	Excellent leaf health (Stay green) and tolerance to stalk lodging
4	COLONEL*	IYADILPRO	✓	✓	✓	–	Excellent leaf health (Stay green), good cob cover and tolerance to stalk lodging
5	P-3966	PIONEER	✓	✓	–	–	Drought tolerance and, grain health
6	ZAPADOR	IYADILPRO	✓	✓	–	–	Drought and cob tolerance and good cob cover
7	TRITON-22	UNISEM	✓	✓	–	–	Tolerant to tar spot complex and to stalk lodging
8	POSEIDON	UNISEM	✓	✓	–	–	Excellent cob coverage and tolerant to stalk lodging
9	N1R03	NOVASEM	✓	✓	–	–	Excellent foliar health (Stay green) and tolerance to stalk lodging
10	MS-404*	MATER SEEDS	–	–	✓	✓	Excellent leaf health (Stay green), good cob cover and tolerance to stalk lodging
11	SKW-502**	REYCOLL	–	–	✓	–	Tolerant to tar spot complex and tolerance to stalk lodging
12	SKW-507**	REYCOLL	–	–	✓	–	High yield and quality of plant and grain
13	SKW-510**	REYCOLL	–	–	✓	–	Tolerant to tar spot complex, stalk lodging and flour quality
14	N-830**	NOVASEM	–	–	✓	–	Excellent grain health, good cob coverage and tolerance to stalk lodging

* Promising hybrids with high yield and low incidence of *A. flavus* and aflatoxins.

** Second group of promising hybrids that need another evaluation to confirm the results.

environment (Zhang et al., 2022).

Applications of the biocontrol product were done superficially when the maize had seven leaves (V7) and continued up to two to three weeks before flowering, marking the end of the vegetative phase (VT), with a rate of 10 kg ha⁻¹. This extended application window allowed for both manual and mechanized application methods, utilizing basic equipment accessible to the farmers themselves (Mauro et al., 2018). In Tamaulipas, applications were conducted between March 1st and April 30th, while in Campeche, they were conducted from August 1st to September 8th. Heat units (HU) were employed to accurately determine the flowering date, irrespective of the planting date. The calculation of HU followed the methodology described previously (Chassaigne-Ricciulli et al., 2020).

2.3. Treatments and experimental design

At each site, side by side experiments were conducted, including two treatments: (i) a biocontrol-treated field and (ii) an untreated control field. Each experiment was carried out in a randomized completed block design (RCBD) with four replicates per treatment (hybrids). Based on the measurements of the land and machinery available to each farmer, the number of rows of each hybrid were distributed in the experimental trials, maintaining a separation of 200–500 m between treatments. Farmers followed their traditional agronomic management practices in both treatments.

2.4. Ear and grain sample collections

From 2019 to 2021, in Tamaulipas and Campeche, manual harvesting was done, and the ears were collected in plastic mesh bags measuring 55 cm × 32 cm. Border plants' ears were deliberately excluded, and at every meter interval, a random cob was harvested in a zigzag pattern between the central rows of each hybrid, until 100 ears were collected (Sserumaga et al., 2020). A random subsample of 10 ears was then selected, placed in cotton bags, and sent to CIMMYT-Textcoco, State of Mexico. Moisture content was adjusted to 11–13 % by placing the ears in the greenhouse for a seven-day drying process. Before shelling, data regarding weight, appearance, and ear condition were recorded. Grain yield averages were obtained using grain weight, number of ears harvested, and plant density per hectare. Yields were adjusted to 14 % moisture. For 2022, three composite samples of approximately 2 kg of grain were taken directly from the threshing on the transport truck every 10 ha, respecting the separation by hybrid. All grain samples with a final moisture content between 11–13 % were stored at 4 °C.

2.5. Ear aspect and severity of *Aspergillus flavus*

The ear aspect was scored on a 1 to 5 scale, where 1 = clean, uniform, large and well-filled ears and 5 = rotten, variable, small and partially filled ears (Dao et al., 2014). Ear rot severity was rated by two methods. The first method used a visual scale of 1 to 7 (1 = 0 %, 2 = 1–5 %, 3 = 6–10 %, 4 = 11–25 %, 5 = 26–50 %, 6 = 51–75 %, and 7 = 76–100 % ear rot damage), as described previously (Reid et al., 1994). The second method was an average percentage (%) of ear rot, out of the total number of ears harvested.

2.6. Aflatoxin quantification in grain samples

The AF levels in grain maize sampled at harvest were determined using NEOGEN's Reveal® Q + MAX method (Reveal Q+ MAX, 2023). The grain from the 10 ears was milled until at least 95 % of the flour passed through a 20-mesh sieve. The flour was homogenized, and two subsamples of 10 ± 0.05 g were taken for shaking in a horizontal Eberbach for 3 min with 50 ml deionized water and Reveal® Q + MAX kit. Subsequently, it was allowed to stand for 10 min and the supernatant was filtered through a filter syringe. From the final solution, 100 µl was taken and mixed with the developer solution from the kit. Finally, a test strip was inserted for 6 min to take the reading on the previously calibrated AccuScan Gold (AccuScan Gold, 2023). The reading was recorded in parts per billion (ppb) and if it was higher than 50 ppb, the solution was diluted 1:6 or as many times as necessary until it could be detected. Total AF concentration data were calculated using the equation: $y = \log_{10}(1 + x)$.

2.7. Statistical analysis

The statistical model considered the four replications as random effects, while the treated and the untreated maize hybrids served as fixed effects. The following traits were considered in all trials: grain yield, ear aspect, severity of ear rot and logarithm of AF concentration. Statistical analysis was performed using R and its complementary packages specialized in mixed models and in marginal means estimation (Bates et al., 2014; Lenth et al., 2023; R Core Team, 2023). Inferences were made by means of the analysis of variance and through the estimation and statistical comparisons of means, contrasts, and interaction effects.

3. Results

3.1. Aflatoxin concentration levels in maize grain from AF36-Prevail® treated and untreated fields

A total of 1,479 grain samples were collected from 23 hybrids and

analyzed across 69 sites in Tamaulipas and Campeche from 2019 to 2022. Of these, 592 samples originated from biocontrol-treated fields and 592 samples from untreated fields during 2019–2021. In 2022, 295 samples were obtained exclusively from treated fields, which included the most promising hybrids with the lowest AF presence and the highest yields (Table 3). The data shows a consistent and notable reduction in AF content, averaging at 66.5 %, in the samples acquired from the treated fields during the years 2019, 2020, and 2021, as compared to samples from the untreated fields (Table 4). Specifically, the AF content in maize from treated fields showed a more substantial reduction in 2021, 76.3 % less in Tamaulipas and 89.9 % less in Campeche. This showcased an incremental positive impact of the biocontrol method, particularly when compared to the initial year of treatment in 2019, where reductions stood at 59.0 % in Tamaulipas and 81.5 % in Campeche. However, during the SS season of 2020 in Campeche, a lower percentage of reduction of AF was observed in AF36-Prevail-treated fields (44.7 %). Notably, that particular year experienced AF concentrations nearly 100 times higher than the other two evaluation years in both treatments. These events can be attributed to irregular rainfall distribution, causing water stress during the maturity stage, leading to heightened AF production (Table 4 and Fig. 1B).

3.2. AF36-Prevail® biocontrol and maize hybrids in the reduction of *A. flavus* and aflatoxins

3.2.1. Tamaulipas

The average incidence of *Aspergillus* ear rot was higher in maize from untreated fields. On a scale of 1 to 7, where 1 represented no ear rot damage and 7 represented complete maximum ear rot damage, the extent of ear rot we observed ranged from 1 to 4 (or 0 % to 25 %). The correlation between ear rot and the presence of AF was weak ($r = 0.36$). Analysis of variance and adjusted means was conducted to assess the impact of treatments (biocontrol-treated and untreated) on yield, ear aspect, severity of *A. flavus*, and AF levels, as well as its simple interactions as fixed in Tamaulipas during the FW2018-19, FW2019-20, and FW2020-21 cycles. The study involved three different hybrids (AG-2525, NB-722, and P-3057). The results revealed that AF levels have significant differences ($p = \leq 0.001$) in terms of treatments and years of evaluation, as well as their interaction between treatments and years

Table 3

Number of maize grain samples from fields treated with the biocontrol (AF36 Prevail®) and from untreated fields (Control) in Tamaulipas and Campeche during 2019–2022.

Year	Location	Region	No. sites	Number of samples*	
				AF36	Control
2019	Tamaulipas	Río Bravo	1	32	32
2019	Campeche	Yalnon	1	36	36
2020	Tamaulipas	Díaz Ordaz	2	48	48
2020	Tamaulipas	Río Bravo	4	96	96
2020	Tamaulipas	Abasolo	2	48	48
2020	Campeche	Yalnon	1	36	36
2021	Tamaulipas	Díaz Ordaz	2	40	40
2021	Tamaulipas	Río Bravo	4	120	120
2021	Tamaulipas	Abasolo	2	100	100
2021	Campeche	Yalnon	1	36	36
2022	Tamaulipas	Díaz Ordaz	4	15	NS**
2022	Tamaulipas	Río Bravo	10	105	NS**
2022	Tamaulipas	Abasolo	7	30	NS**
2022	Campeche	Yalnon	12	50	NS**
2022	Campeche	Chavi	1	15	NS**
2022	Campeche	Katab	5	15	NS**
2022	Campeche	Emiliano Zapata	3	15	NS**
2022	Campeche	Nuevo Progreso	2	20	NS**
2022	Campeche	Santa Rosa	5	30	NS**

* In 2019, 2020 and 2021, pre-harvest ear samples were collected. For 2022, grain samples were collected directly from the threshing machine.

** NS = No Samples.

(Table 5). However, there was no significant difference ($p = 0.578$) among hybrids with respect to AF levels, because the hybrids with the lowest AF levels were filtered out during the years of evaluation. Regarding grain yields, significant differences ($p = \leq 0.001$) were observed for both hybrids and years of evaluation and interactions between hybrids and years and between years and treatments. Therefore, it can be said that the hybrids have significant differences in grain yield, with hybrid NB722 being the best, followed by AG2525 and then P3057 (Table 6). This indicates that hybrid selection for yields and treatments with and without AF36 for AF levels in Tamaulipas had a significant effect on these parameters.

3.2.2. Campeche

The average incidence of *Aspergillus* ear rot was also higher in maize from untreated fields at Campeche. There was no correlation ($r = 0.08$) between ear rot and the presence of AF. Analysis of variance and adjusted means for Campeche, during the FW2019-20, FW2020-21 and FW2021-22 cycles, revealed that AF levels had significant differences ($p = \leq 0.001$) for years of evaluation and the interaction between years and hybrids (Table 7). Regarding grain yields, significant differences ($p = \leq 0.001$) were observed for both hybrids and years of evaluation and the interaction between hybrids and years. The hybrids CORONEL and P-4279 were the best in yield, followed by P-4028 and then TORNADO (Table 8). Although the mean AF levels in the biocontrol treatment were lower than in the untreated maize, the difference was not statistically reliable because the years of evaluation data were not homogeneous.

4. Discussion

During the four-year evaluation study (2019–2022), we employed an integrated management approach that combined AF36-Prevail® biocontrol application with AF resistant maize germplasm adapted to the regions of Tamaulipas and Campeche, to mitigate AF contamination. The data showed a consistent and notable reduction in AF content, with an average reduction of 66.5 %, in samples from fields treated with biocontrol compared to samples from untreated fields. Regarding maize germplasm, 26 % of the 23 hybrids evaluated between 2019–2021 showed significant improvements in yield and tolerance to AF, which were planted on 500 to 2,000 ha in Tamaulipas and Campeche, respectively, during 2022. For Tamaulipas, the hybrids NB-722, AG-2525, and P-3057 were identified as having better adaptation to the climatic conditions of the region, better ear coverage, higher yield, and lowest AF contamination (< 20 ppb). Similarly, for Campeche, the hybrids CORONEL, P-4028, P-4279, and TORNADO demonstrated resilience to water stress and reduced AF levels.

In Tamaulipas, a strong relationship ($r = 0.90$) was observed between planting date and the duration of male and female flowering periods. In other words, December and January plantings had about 100 days of flowering compared to February plantings, which were reduced to 85 days of flowering. But March plantings, when temperatures increased, had shorter cycles, up to 65 days to flowering. However, the correlation ($r = 0.27$) between sowing dates and AF levels was very weak. This is because in Tamaulipas relief irrigation is done during grain filling or maturity to avoid stress on the plants. Unlike Campeche, where the occurrence of AF was significantly influenced by rainfall patterns. In seasons with well-distributed rainfall throughout the crop development stage, lower AF concentrations were observed. However, when water stress occurs during the maturity stage, higher AF levels were detected. This was particularly evident in the case of Campeche SS2020, as indicated in Table 4. In SS2022, a strong relationship ($r = 0.47$) was observed between planting dates and AF prevalence. This implies that there was a trend toward reduced AF levels in early plantings, specifically from June 20 to July 15. These findings suggest that early planting of maize within this period can potentially contribute to decreased AF contamination in Campeche. In addition, the site where biocontrol was applied consistently for, four consecutive years, had the lowest AF

Table 4

Total aflatoxin concentrations in maize grain samples from fields treated with biocontrol (AF36 Prevail®) and from untreated fields (control) in Tamaulipas and Campeche during 2019, 2020 and 2021.

Location	Region	Year	Aflatoxin concentrations (ppb)								Reduction (%) ^{**}
			Control				AF36				
			Min	Max	SD	Mean*	Min	Max	SD	Mean*	
Tamaulipas	Río Bravo	2019	2.5	464.0	101.6	75.6	2.8	98.0	27.0	31.0	59.0
	Díaz Ordaz	2020	3.4	1465.0	364.0	261.0	2.5	1724.0	329.6	118.3	54.7
	Río Bravo	2020	1.6	4020.0	608.6	287.6	1.7	3572.5	424.5	142.1	50.6
	Abasolo	2020	1.2	1350.0	242.2	79.4	1.6	712.0	109.7	30.4	61.8
	Díaz Ordaz	2021	2.8	1960.0	318.8	80.5	2.5	112.6	25.2	17.3	78.5
	Río Bravo	2021	0.0	2296.0	370.6	136.2	1.1	1248.0	123.1	24.0	82.4
Campeche	Abasolo	2021	0.0	3750.0	489.1	98.3	0.0	825.0	142.6	37.6	61.8
	Yalnón	2019	1.9	570.0	159.0	94.6	2.3	218.0	41.0	17.5	81.5
	Yalnón	2020	5.6	4405.0	918.4	664.8	3.4	1942.5	518.4	368.0	44.7
	Yalnón	2021	0.0	232.8	39.9	9.8	0.0	2.6	0.8	1.0	89.9

* The means of the aflatoxin values.

** The percentage reduction was calculated for each region in each year and location as follows: (mean of control – mean of AF36 treatment)/mean of control × 100.

Table 5

Analysis of variance for yield, ear aspect, ear rot rate, ear rot percent, and aflatoxin evaluations in Tamaulipas. Three hybrids (AG-2525, NB-722 and P-3057), evaluated under two treatments (AF36 Prevail® and control) *. F-Snedecor test for the fixed terms' mean square variations against the residuals' variance component ** and respective p value given the Satterthwaite degrees of freedom adjustment.

	Yield		Ear aspect		Ear rot rate		Ear rot percent		Aflatoxin	
	F test	p value	F test	p value	F test	p value	F test	p value	F test	p value
Hybrids	11.449	<0.001	13.250	<0.001	129.463	<0.001	5.073	0.007	0.549	0.578
Treatments	0.448	0.505	0.031	0.861	0.193	0.661	0.142	0.707	14.092	<0.001
Treatments x Hybrids	0.786	0.457	2.198	0.113	1.161	0.315	3.194	0.043	0.272	0.762
Years	47.338	<0.001	14.924	<0.001	11.846	<0.001	12.222	<0.001	8.646	<0.001
Years x Hybrids	2.771	<0.001	2.686	<0.001	3.609	<0.001	2.335	<0.001	2.271	<0.001
Years x Treatments	3.330	<0.001	0.596	0.880	0.937	0.530	0.675	0.812	2.108	0.013
Residual	1.365		0.141		0.124		47.480		0.373	

* Experimental Design: Trials consisting of two (AF36 Prevail® and control treatments) side bade side RCBD experiments evaluations three hybrids (AG-2525, NB-722 and P-3057) with four replicates over three years (2018–19, 2019–20 and 2020–21) over three sites of Tamaulipas State, MX.

** Statistical Model: Linear mixed model having sources (hybrids, treatments, and trials) as well as its simple interactions as fixed and blocks within experiments over trials as random. Parameters estimated by restricted (residual) maximum likelihood (reml).

Table 6

Adjusted Means for yield, ear aspect, ear rot rate, ear rot percent, and aflatoxin evaluations in Tamaulipas. Three hybrids (AG-2525, NB-722 and P-3057), evaluated under two treatments (AF36 Prevail® and control) *. The marginal and interaction least square means (mean), given residuals' variance component ** and respective standard error of the estimation (se).

		Yield		Ear aspect		Ear rot rate		Ear rot percent		Aflatoxin	
		mean	se	mean	se	mean	se	mean	se	mean	se
<i>marginals</i>											
Hybrids	AG-2525	9.529	0.103	1.283	0.032	2.228	0.035	5.361	0.654	2.082	0.057
	NB-722	9.937	0.103	1.246	0.032	2.382	0.035	4.997	0.654	2.153	0.057
	P-3057	9.264	0.103	1.452	0.032	2.926	0.035	7.462	0.654	2.145	0.057
Treatments	AF36	9.535	0.089	1.324	0.030	2.522	0.032	6.106	0.624	1.987	0.053
	Control	9.619	0.089	1.331	0.030	2.502	0.032	5.774	0.624	2.267	0.053
<i>interactions</i>											
AF36	AG-2525	9.474	0.146	1.228	0.046	2.250	0.049	4.316	0.925	1.954	0.080
	NB-722	9.814	0.146	1.265	0.046	2.419	0.049	5.895	0.925	1.981	0.080
	P-3057	9.317	0.146	1.478	0.046	2.897	0.049	8.107	0.925	2.025	0.080
control	AG-2525	9.584	0.146	1.338	0.046	2.206	0.049	6.405	0.925	2.210	0.080
	NB-722	10.060	0.146	1.228	0.046	2.346	0.049	4.099	0.925	2.324	0.080
	P-3057	9.211	0.146	1.426	0.046	2.956	0.049	6.817	0.925	2.266	0.080

* Experimental Design: Trials consisting of two (AF36 Prevail® and control treatments) side bade side RCBD experiments evaluations three hybrids (AG-2525, NB-722 and P-3057) with four replicates over three years (2018–19, 2019–20 and 2020–21) over three sites of Tamaulipas State, MX.

** Statistical Model: Linear mixed model having sources (hybrids, treatments, and trials) as well as its simple interactions as fixed and blocks within experiments over trials as random. Parameters estimated by restricted (residual) maximum likelihood (reml).

levels, with an average concentration of 10.1 ppb.

Temperature, precipitation, and relative humidity data were obtained from weather stations in the target regions (CONAGUA, 2023; Weather-Spark, 2023). High daytime and nighttime temperatures coincide with the flowering stage in both Tamaulipas and Campeche

according to crop phenology. However, while rainfall increased in Tamaulipas, water stress has occurred in Campeche (Fig. 1). Accurately determining the flowering date using heat units (HU) (Chassaing-Ricciulli et al., 2020) was critical in this study for timing biocontrol applications, independent of planting date, genotype, and region.

Table 7

Analysis of variance for yield, ear aspect, ear rot rate, ear rot percent, and aflatoxin evaluations in Campeche. Four hybrids (CORONEL, P-4028, P-4279 and TORNADO), evaluated under two treatments (AF36 Prevail® and control) *. F-Snedecor test for the fixed terms' mean square variations against the residuals' variance component ** and respective p value given the Satterthwaite degrees of freedom adjustment.

	Yield		Ear aspect		Ear rot rate		Ear rot percent		Aflatoxin	
	F test	p value	F test	p value	F test	p value	F test	p value	F test	p value
Hybrids	6.657	<0.001	0.799	0.498	1.031	0.385	1.809	0.155	1.321	0.276
Treatments	1.037	0.322	2.368	0.128	0.652	0.430	2.617	0.123	1.373	0.257
Treatments x Hybrids	1.030	0.386	0.612	0.609	1.281	0.289	3.259	0.028	0.551	0.649
Years	38.482	<0.001	2.368	0.100	1.748	0.202	2.619	0.100	49.081	<0.001
Years x Hybrids	5.731	<0.001	3.377	0.005	1.219	0.309	1.268	0.286	5.733	<0.001
Years x Treatments	1.790	0.195	0.687	0.506	0.496	0.617	0.991	0.390	1.287	0.300
Residual	0.194		0.186		0.333		4.934		0.358	

* Experimental Design: Trials consisting of two (AF36 Prevail® and control treatments) side bade side RCBD experiments evaluations four hybrids (CORONEL, P-4028, P-4279 and TORNADO) with four replicates over three years (2019, 2020 and 2021) over three sites of Campeche State, MX.

** Statistical Model: Linear mixed model having sources (hybrids, treatments, and trials) as well as its simple interactions as fixed and blocks within experiments over trials as random. Parameters estimated by restricted (residual) maximum likelihood (reml).

Table 8

Adjusted means for yield, ear aspect, ear rot rate, ear rot percent, and aflatoxin evaluations in Campeche. Four hybrids (CORONEL, P-4028, P-4279 and TORNADO), evaluated under two treatments (AF36 Prevail® and control) *. The marginal and interaction least square means (mean), given residuals' variance component ** and respective standard error of the estimation (se).

		Yield		Ear aspect		Ear rot rate		Ear rot percent		Aflatoxin	
		mean	se	mean	se	mean	se	mean	se	mean	se
<i>marginals</i>											
Hybrids	CORONEL	5.782	0.102	2.771	0.088	1.750	0.121	1.996	0.457	2.099	0.140
	P-4028	5.431	0.102	2.854	0.088	1.792	0.121	2.248	0.457	2.148	0.140
	P-4279	5.519	0.102	2.958	0.088	1.750	0.121	2.265	0.457	1.963	0.140
	TORNADO	5.223	0.102	2.896	0.088	2.000	0.121	3.364	0.457	1.837	0.140
Treatments	AF36	5.556	0.093	2.802	0.062	1.875	0.091	2.845	0.330	1.904	0.129
	control	5.421	0.093	2.938	0.062	1.771	0.091	2.091	0.330	2.119	0.129
<i>interactions</i>											
AF36	CORONEL	5.897	0.144	2.750	0.124	2.000	0.171	2.844	0.646	1.995	0.198
	P-4028	5.598	0.144	2.708	0.124	1.750	0.171	2.777	0.646	2.006	0.198
	P-4279	5.543	0.144	2.958	0.124	1.750	0.171	1.444	0.646	1.979	0.198
	TORNADO	5.186	0.144	2.792	0.124	2.000	0.171	4.316	0.646	1.638	0.198
control	CORONEL	5.668	0.144	2.792	0.124	1.500	0.171	1.148	0.646	2.203	0.198
	P-4028	5.264	0.144	3.000	0.124	1.833	0.171	1.719	0.646	2.289	0.198
	P-4279	5.494	0.144	2.958	0.124	1.750	0.171	3.086	0.646	1.947	0.198
	TORNADO	5.260	0.144	3.000	0.124	2.000	0.171	2.413	0.646	2.035	0.198

* Experimental Design: Trials consisting of two (AF36 Prevail® and control treatments) side bade side RCBD experiments evaluations four hybrids (CORONEL, P-4028, P-4279 and TORNADO) with four replicates over three years (2019, 2020 and 2021) over three sites of Campeche State, MX.

** Statistical Model: Linear mixed model having sources (hybrids, treatments, and trials) as well as its simple interactions as fixed and blocks within experiments over trials as random. Parameters estimated by restricted (residual) maximum likelihood (reml).

Developing an integrated epidemiological model based on meteorological data to evaluate and predict AF contamination risks would enable farmers to make informed decisions based on expected AF levels, thereby mitigating risks across the supply chain from planting to grain delivery (Stutt et al., 2023). Implementing such a model would require more granular climate data, collected directly from individual plots.

Predictions for the current century (2001–2100), indicate a global temperature increase between 1.4 and 5.8 °C, which will affect crop development and their adaptive capacity, as well as alter (possibly by expansion) along with changes in the current distribution and densities of AF-producing fungi (Arora, 2019; Bidartondo et al., 2018). Maize germplasm should be well adapted to the climatic conditions of any given region, with tolerance to drought, insects, and native pathogens. It has been reported that maize cultivars grown outside their adaptive range and without resistance or tolerance to ear rot are more susceptible to AF (Xu et al., 2022). Susceptibility to *A. flavus* infection and AF production in the plant occurs as part of a survival mechanism between oxidative stress that is enhanced by the effects of heat and drought, during sensitive periods of flowering and grain development that vary from year to year (Fountain et al., 2020; Gruber-Dorninger et al., 2019). In Campeche, southern Mexico, no significant differences between

treatments were observed (Table 7). Ear rot and AF contamination were significantly higher in maize from untreated fields (Table 4). The reduced levels of ear rot and AF in fields treated with biocontrol may be attributed to i) the biocontrol agent modulating and decreasing the population of toxigenic *A. flavus*, and ii) AF36's limited ability to cause rot in maize, functioning primarily by displacing toxigenic strains. Agronomic practices were consistent across both treatments, following local farming protocols. Consequently, the observed changes in ear rot and AF levels may have been influenced by annual climatic variability, farmer-specific management practices, and the pathogen's population dynamics in the soil. Further research should focus on establishing a definitive cause-and-effect relationship through comprehensive morphological and molecular analyses of soil in treated and untreated fields to elucidate the population structure of *A. flavus* before and after biocontrol application.

Plant resistance to AF is a quantitative trait with relatively low heritability that interacts significantly with the effects of the environment in each region (Warburton et al., 2011). These resistance mechanisms are governed by multiple genes in an interaction of numerous antioxidant proteins and enzymes, suggesting that additive gene effects are more important than dominant gene effects (Fountain et al., 2014;

Soni et al., 2020). Most resistance breeding programs are in the private sector, but these have not made significant efforts to develop AF resistant maize. On the other hand, public sector breeding programs have made significant progress in improving resistance to *Aspergillus* ear rot (Womack et al., 2020). The current study identified hybrids with acceptable yields and reduced susceptibility to AF contamination, particularly when biocontrol was applied. The one potential drawback to using the AF36 strain as a biocontrol agent is its production of another well-known mycotoxin known as cyclopiazonic acid or CPA (Abbas et al., 2011). However, there are no regulations against the presence of CPA in food or feed products although some suggest there should be (Burdock and Flamm, 2000). Future research should evaluate new maize hybrids from seed companies and regional growers to find the more resilient, resistant, and high-yielding genotypes. Some of the AF susceptible hybrids we discarded, in evaluation years 2020 and 2021, had good yield capacity and could still be used by growers if treated with an effective biocontrol, should one become commercially available in Mexico. Continuing evaluations with new hybrids that exhibit traits like premature ear drying (Stay Green), strong stalks, excellent ear coverage, and tolerance to abiotic stressors like drought and heat is essential to ensure resilient, resistant, and high-yielding maize meeting quality and safety requirements.

The biosynthesis of AF is costly and very stressful for the fungi that produce it (Calvo et al., 2002). For the fungus to synthesize AF, it requires the activation of 30 genes and 20 enzymatic reactions (Yu, 2012). Genes in the AF gene cluster can be affected by different types of mutations, including substitutions, insertions and deletions that result in the non-aflatoxigenic phenotype. For instance, AF36-Prevail®, Afla-Guard® and AF-X1®, are single-strain formulations *A. flavus* strain. The strain AF36 (NRRL18543) has a single nucleotide polymorphism (SNP) mutation located at nucleotide 591 in its *aflC* gene, that prematurely introduces a stop codon and halts AF biosynthesis (Ehrlich and Cotty, 2004). The component strain in Afla-Guard® (NRRL21882) lacks the entire AF biosynthesis gene cluster (Chang et al., 2005). Aflasafe, has also been developed with the mixture of several endemic strains with different types of gene. There have been different types of Aflasafe developed, tailored to the country of use, throughout Africa, with the objectives of extending adaptation to different soil types, microclimates, native microbial competition and establishing a broad population of non-aflatoxigenic strains in the medium and long term for protection mainly in maize and peanut crops (Moral et al., 2020). These strains outcompete native AF producing fungi for resources and expedite regulatory approval compared to exotic fungi (Moral et al., 2020). Future research should focus on the distribution of toxigenic and non-aflatoxigenic *A. flavus* strains in maize fields in tropical and subtropical Mexico to identify additional biocontrol strains that are equal to or better than AF36-Prevail® at controlling AF.

Integrated AF management, including fertilization, irrigation, crop rotation, pest control, and best post-harvest practices, is crucial for comprehensive protection (Logrieco et al., 2021; Munkvold, 2014). This study showed that biocontrol application across varied locations with different maize hybrids and was good agricultural practice for the reduction of AF levels. Although the need for AF management extends to post-harvest and storage, reducing field contamination is a key step in minimizing AF contamination worldwide, benefiting both consumer health and farmer economies. Research should prioritize the development of biocontrol products with endemic strains to enhance their success and persistence in treated fields. This study underscores that maize germplasm alone is insufficient to combat AF contamination. Moreover, post-harvest, transport, storage, and processing practices must be optimized to prevent fungal growth and AF production, as poor management during these stages can significantly increase AF levels.

5. Conclusion

The current study demonstrated that the commercial biocontrol

product AF36 Prevail® effectively reduced AF to levels below 20 ppb in maize fields in Tamaulipas and Campeche, Mexico. This was achieved in combination with regionally adapted hybrid that show inherent resistance to AF contamination, as well as good ear coverage as well as selecting drought tolerance, and optimal sowing dates. It is recommended to continue evaluating new market hybrids and testing native non-aflatoxigenic strains of *A. flavus* with comparable or superior biocontrol activity to AF36-Prevail®. This approach, along with good harvest and post-harvest practices, can enhance the effectiveness of biocontrol efforts.

Author Contributions

NPR supervised all experiments. CMZ performed the practical work and completed the experiments, AMM and LCS performed aflatoxin quantification in grain samples, ETM provided help during field experiments. SH assisted in data analysis and interpretation. CMZ and NPR wrote the initial version of the manuscript. All authors reviewed the manuscript and agreed with the publication of the paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Natalia Palacios Rojas reports financial support, administrative support, and statistical analysis were provided by CGIAR Consortium. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding sources

This research was conducted thanks to the financial support of CGIAR Plant Health Initiative and the project on sustainable grain production from Grupo Maseca (GRUMA). Any opinions, findings, conclusion or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of CGIAR Research or its funders.

Declaration of generative AI and AI-assisted technologies in the writing process.

During the preparation of this work the author(s) used ChatGPT in order to assist with language and grammar editing. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRedit authorship contribution statement

Carlos Muñoz-Zavala: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Aide Molina-Macedo:** Writing – review & editing, Methodology, Investigation, Data curation. **Fernando H. Toledo:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Eugenio Telles-Mejía:** Writing – review & editing, Methodology, Investigation, Data curation. **Luisa Cabrera-Soto:** Writing – review & editing, Methodology, Investigation. **Natalia Palacios-Rojas:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Natalia Palacios Rojas reports financial support, administrative support, and statistical analysis were provided by CGIAR Consortium. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Acknowledgments

The authors thank the farmers for their availability and collaboration during this study. They also express gratitude to the national and multinational seed companies that donated the evaluated seeds and to SENASICA for the corresponding permits to conduct this research. We thank INIFAP and SADER (Mexico) for their support. Special thanks go to colleagues Alberto Cabello Cortes, Jesus Perez Gómez, Eduardo Tovar López, and Kai Sonder from CIMMYT (Mexico) and colleagues from the agriculture team from GRUMA for their support, especially Ignacio Castañeda and Mario Farfan Dominguez. Appreciation is also expressed to Alejandro Ortega-Beltran and Ranajit Bandyopadhyay from IITA (Nigeria) for their advice, expertise, and collaboration.

References

- Abbas, H.K., Zablotowicz, R.M., Horn, B.W., Phillips, N.A., Johnson, B.J., Jin, X., Abel, C. A., 2011. Comparison of major biocontrol strains of non-aflatoxigenic *Aspergillus flavus* for the reduction of aflatoxins and cyclopiiazonic acid in maize. *Food Addit. Contam.: Part A* 28 (2), 198–208. <https://doi.org/10.1080/19440049.2010.544680>.
- AccuScan Gold. (2023). NEOGEN® introduces Reveal® Q+ MAX. https://www.neogen.com/4adbe6/globalassets/pim/assets/original/10022/official_8085_reveal-q-plus-aflatoxin_procedures-gold_en-us.pdf (last accessed 03 December 2024).
- Arora, N.K., 2019. Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability* 2 (2), 95–96. <https://doi.org/10.1007/s42398-019-00078-w>.
- Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., Senghor, A.L., Adhikari, B.N., Cotty, P.J., 2016. Biological control of aflatoxins in Africa: Current status and potential challenges in the face of climate change. *World Mycotoxin J.* 9 (5), 771–789. <https://doi.org/10.3920/WMJ2016.2130>.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4 (arXiv:1406.5823). *arXiv*. doi: 10.48550/arXiv.1406.5823.
- Bhandari, K.B., Longing, S.D., West, C.P., 2020. Soil microbial communities in corn fields treated with atoxigenic *Aspergillus flavus*. *Soil Systems* 4 (2), 2. <https://doi.org/10.3390/soilsystems4020035>.
- Bidartondo, M.I., Ellis, C., Kausrud, H., Kennedy, P.G., Lilleskov, E., Suz, L., Andrew, C., 2018. Climate change: fungal responses and effects State of the World's. *Fungi* 62–69.
- Burdock, G.A., Flamm, W.G., 2000. Review article: safety assessment of the mycotoxin cyclopiiazonic acid. *Int. J. Toxicol.* 19 (3), 195–218. <https://doi.org/10.1080/10915810050074964>.
- Calvo, A.M., Wilson, R.A., Bok, J.W., Keller, N.P., 2002. Relationship between secondary metabolism and fungal development. *Mycobiol. Mol. Biol. Rev.* 66 (3), 447–459. <https://doi.org/10.1128/mmr.66.3.447-459.2002>.
- Chang, P.-K., Horn, B.W., Dorner, J.W., 2005. Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates. *Fungal Genetics and Biology* 42 (11), 914–923. <https://doi.org/10.1016/j.fgb.2005.07.004>.
- Chassaigne-Ricciulli, A.A., Mendoza-Onofre, L.E., Córdova-Téllez, L., Carballo-Carballo, A., San Vicente-García, F.M., Dhliwayo, T., 2020. Development of seed production technology of CIMMYT tropical single cross maize hybrids. *Agriculture* 10 (7), 7. <https://doi.org/10.3390/agriculture10070259>.
- Collinge, D.B., Jensen, D.F., Rabiey, M., Sarrocco, S., Shaw, M.W., Shaw, R.H., 2022. Biological control of plant diseases—What has been achieved and what is the direction? *Plant Pathol.* 71 (5), 1024–1047. <https://doi.org/10.1111/ppa.13555>.
- CONAGUA. (2023). Comisión Nacional del Agua. <http://smn.cna.gob.mx/es/> (last accessed 03 December 2024).
- Cotty, P.J., 1997. Aflatoxin-producing potential of communities of *Aspergillus* section *Flavi* from cotton producing areas in the United States. *Mycol. Res.* 101 (6), 698–704. <https://doi.org/10.1017/S0953756296003139>.
- Cotty, P.J., 2006. Biocompetitive exclusion of toxigenic fungi. In: *The Mycotoxin Factbook*. Wageningen Academic, pp. 179–197. https://doi.org/10.3920/9789086865871_011.
- Dao, A., Sanou, J., Gracen, V., Danquah, E.Y., 2014. Heterotic relationship between INERA, CIMMYT and IITA Maize Inbred Lines under Drought and Well-Watered Conditions 201–210.
- Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N., & Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in humans and animals. In R. G. Guevara-Gonzalez (Ed.), *Aflatoxins—Biochemistry and Molecular Biology*. *InTech*. doi: 10.5772/22717.
- Díaz de León-Martínez, L., Rodríguez-Aguilar, M., Wong-Arce, A., Díaz-Barriga, F., Bañuelos-Hernández, B., Rosales-Mendoza, S., Flores-Ramírez, R., 2020. Evaluation of acute and chronic exposure to aflatoxin B1 in indigenous women of the Huasteca Potosina, Mexico. *Environ. Sci. Pollut. Res.* 27 (24), 30583–30591. <https://doi.org/10.1007/s11356-020-09361-4>.
- Díaz-Franco, A., Montes-García, N., 2008. La fitopatología en la región semiárida de Tamaulipas, México: Resena histórica. *Revista Mexicana De Fitopatología* 26 (1), 62–70. https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0185-33092008000100010.
- Dorner, J.W., 2010. Efficacy of a biopesticide for control of aflatoxins in corn. *J. Food Prot.* 73 (3), 495–499. <https://doi.org/10.4315/0362-028x-73.3.495>.
- Espinosa, E.T., Askar, K.A., Naccha Torres, L.R., Olvera, R.M., Santa Anna, J.P.C., 1995. Quantification of aflatoxins in corn distributed in the city of Monterrey, Mexico. *Food Addit. Contam.* 12 (3), 383–386. <https://doi.org/10.1080/02652039509374319>.
- Fountain, J.C., Pandey, A.K., Nayak, S.N., Bajaj, P., Wang, H., Kumar, V., Chitikineni, A., Abbas, H.K., Scully, B.T., Kemerait, R.C., Pandey, M.K., Guo, B., Varshney, R.K., 2020. Transcriptional responses of toxigenic and atoxigenic isolates of *Aspergillus flavus* to oxidative stress in aflatoxin-conductive and non-conductive media. *World Mycotoxin J.* 13 (4), 443–457. <https://doi.org/10.3920/WMJ2020.2566>.
- Fountain, J., Scully, B., Ni, X., Kemerait, R., Lee, D., Chen, Z.-Y., Guo, B., 2014. Environmental influences on maize-*Aspergillus flavus* interactions and aflatoxin production. *Front. Microbiol.* 5, 40. <https://www.frontiersin.org/articles/10.3389/fmicb.2014.00040>.
- Gruber-Dorninger, C., Jenkins, T., Schatzmayr, G., 2019. Global mycotoxin occurrence in feed: a ten-year survey. *Toxins* 11 (7), 375. <https://doi.org/10.3390/toxins11070375>.
- Grubisha, L.C., Cotty, P.J., 2015. Genetic analysis of the *Aspergillus flavus* vegetative compatibility group to which a biological control agent that limits aflatoxin contamination in US crops belongs. *Appl. Environ. Microbiol.* 81 (17), 5889–5899. <https://doi.org/10.1128/AEM.00738-15>.
- Horn, B.W., Gell, R.M., Singh, R., Sorensen, R.B., Carbone, I., 2016. Sexual reproduction in *Aspergillus flavus* sclerotia: acquisition of novel alleles from soil populations and uniparental mitochondrial inheritance. *PLoS One* 11 (1), e0146169. <https://doi.org/10.1371/journal.pone.0146169>.
- Huang, C., Jha, A., Sweany, R., DeRobertis, C., Damann Jr, K.E., 2011. Intraspecific aflatoxin inhibition in *Aspergillus flavus* is thigmoregulated, independent of vegetative compatibility group and is strain dependent. *PLoS One* 6 (8), e23470. <https://doi.org/10.1371/journal.pone.0023470>.
- INEGI. (2023). Instituto Nacional de Estadísticas Geográficas. <https://www.inegi.org.mx> (last accessed 03 December 2024).
- Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Míguez, F., Riebl, H., & Singmann, H. (2023). emmeans: Estimated Marginal Means, aka Least-Squares Means (Version 1.8.6) [Computer software]. <https://cran.r-project.org/web/packages/emmeans/index.html> (last accessed 03 December 2024).
- Lewis, L., Onsongo, M., Njapau, H., Schurz, Rogers Helen, Lubner, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., DeCock, K., Rubin, C., 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environ. Health Perspect.* 113 (12), 1763–1767. <https://doi.org/10.1289/ehp.7998>.
- Lino-Silva, L.S., Lajous, M., Brochier, M., Santiago-Ruiz, L., Melchor-Ruan, J., Xie, Y., Wang, M., Wu, D., Higson, H., Jones, K., Romero-Martínez, M., Villalpando, S., Mohar, A., Smith, J.W., Alvarez, C.S., McGlynn, K.A., Dean, M., Groopman, J., 2022. Aflatoxin levels and prevalence of TP53 aflatoxin-mutations in hepatocellular carcinomas in Mexico. *Salud Pública De México* 64 (1), 30. <https://doi.org/10.21149/13189>.
- Logrieco, A., Battilani, P., Leggieri, M.C., Jiang, Y., Haesaert, G., Lanubile, A., Mahuku, G., Mesterházy, A., Ortega-Beltran, A., Pasti, M., Smetu, I., Torres, A., Xu, J., Munkvold, G., 2021. Perspectives on global mycotoxin issues and management from the MycoKey Maize Working Group. *Plant Dis.* 105 (3), 525–537. <https://doi.org/10.1094/PDIS-06-20-1322-FE>.
- Mahato, D.K., Lee, K.E., Kamle, M., Devi, S., Dewangan, K.N., Kumar, P., Kang, S.G., 2019. Aflatoxins in food and feed: An overview on prevalence, detection and control strategies. *Front. Microbiol.* 10, 2266. <https://www.frontiersin.org/articles/10.3389/fmicb.2019.02266>.
- Mauro, A., García-Cela, E., Pietri, A., Cotty, P.J., Battilani, P., 2018. Biological control products for aflatoxin prevention in Italy: commercial field evaluation of atoxigenic *Aspergillus flavus* active ingredients. *Toxins* 10 (30), 1. <https://doi.org/10.3390/toxins10010030>.
- Mehl, H.L., Cotty, P.J., 2013. Nutrient environments influence competition among *Aspergillus flavus* genotypes. *Appl. Environ. Microbiol.* 79 (5), 1473–1480. <https://doi.org/10.1128/AEM.02970-12>.
- Méndez-Albores, J.A., Arámbula-Villa, G., Preciado-Ortiz, R.E., Moreno-Martínez, E., 2004. Aflatoxins in pozol, a nixtamalized, maize-based food. *Int. J. Food Microbiol.* 94 (2), 211–215. <https://doi.org/10.1016/j.ijfoodmicro.2004.02.009>.
- Miller, J.D., 2023. Chapter 4 - mycotoxins: still with us after all these years. In: Knowles, M.E., Anelich, L.E., Boobis, A.R., Popping, B. (Eds.), *Present Knowledge in Food Safety*. Academic Press, pp. 62–78. <https://doi.org/10.1016/B978-0-12-819470-6.00009-3>.
- Monge, A., Romero, M., Groopman, J.D., McGlynn, K.A., Santiago-Ruiz, L., Villalpando-Hernández, S., Mannan, R., Burke, S.M., Remes-Troche, J.M., Lajous, M., 2023. Aflatoxin exposure in adults in southern and eastern Mexico in 2018: A descriptive study. *Int. J. Hyg. Environ. Health* 253, 114249. <https://doi.org/10.1016/j.ijheh.2023.114249>.
- Montes, G.N., Reyes, M.C.A., Montes, R.N., Cantu, A.M.A., 2009. Incidence of potentially toxigenic fungi in maize (*Zea mays* L.) grain used as food and animal feed. *CyTA - Journal of Food* 7 (2), 119–125. <https://doi.org/10.1080/19476330902940432>.
- Moore, G.G., Lebar, M.D., Carter-Wientjes, C.H., 2022. Cumulative effects of non-aflatoxigenic *Aspergillus flavus* volatile organic compounds to abate toxin production by mycotoxigenic aspergilli. *Toxins* 14 (5), 340. <https://doi.org/10.3390/toxins14050340>.
- Moral, J., Garcia-Lopez, M.T., Camiletti, B.X., Jaime, R., Michailides, T.J., Bandyopadhyay, R., Ortega-Beltran, A., 2020. Present status and perspective on the future use of aflatoxin biocontrol products. *Agronomy* 10 (4), 491. <https://doi.org/10.3390/agronomy10040491>.

- Moreno, M.E., Gil, G.M., 1991. La biología de *Aspergillus flavus* y la producción de aflatoxinas. Coordinación de La Investigación Científica. Programa Universitario de Alimentos, Universidad Nacional Autónoma de México, México, DF, p. 42.
- Munkvold, G., 2014. Crop management practices to minimize the risk of mycotoxins contamination. In: Maize, T.-Z., Leslie, J.F., Logrieco, A.F. (Eds.), *Mycotoxin Reduction in Grain Chains*. John Wiley & Sons, Ltd, pp. 59–77. <https://doi.org/10.1002/9781118832790.ch5>.
- Richard, J.L., 2006. Chapter 2 - Mycotoxins and mycotoxicoses: A 2004 update. In: Njapau, H., Trujillo, S., van Egmond, H.P., Park, D.L. (Eds.), *Mycotoxins and Phycotoxins. Advances in Determination, Toxicology and Exposure Management. International Journal of Food Microbiology*, pp. 19–30. https://doi.org/10.3920/9789086865857_002.
- Odjo, S., Alakonya, A.E., Rosales-Nolasco, A., Molina, A.L., Muñoz, C., Palacios-Rojas, N., 2022. Occurrence and postharvest strategies to help mitigate aflatoxins and fumonisins in maize and their co-exposure to consumers in Mexico and Central America. *Food Control* 138, 108968. <https://doi.org/10.1016/j.foodcont.2022.108968>.
- Ortega-Beltran, A. (2012). Ecology, distribution, toxigenicity and diversity of aflatoxin-producing fungal communities in maize fields of Mexico and interactions of these fungi with native maize landraces. <https://repository.arizona.edu/handle/10150/265833> (last accessed 03 December 2024).
- Ortega-Beltran, A., Callicott, K.A., Cotty, P.J., 2020. Founder events influence structures of *Aspergillus flavus* populations. *Environ. Microbiol.* 22 (8), 3522–3534. <https://doi.org/10.1111/1462-2920.15122>.
- Ortega-Beltran, A., Cotty, P.J., 2018. Frequent shifts in *Aspergillus flavus* populations associated with maize production in Sonora, Mexico. *Phytopathology* 108 (3), 412–420. <https://doi.org/10.1094/PHYTO-08-17-0281-R>.
- Ortega-Beltran, A., Grubisha, L.C., Callicott, K.A., Cotty, P.J., 2016. The vegetative compatibility group to which the US biocontrol agent *Aspergillus flavus* AF36 belongs is also endemic to Mexico. *J. Appl. Microbiol.* 120 (4), 986–998. <https://doi.org/10.1111/jam.13047>.
- Ortega-Beltran, A., Jaime, R., Cotty, P.J., 2015. Aflatoxin-producing fungi in maize field soils from sea level to over 2000 masl: a three year study in Sonora, Mexico. *Fungal Biology* 119 (4), 191–200. <https://doi.org/10.1016/j.funbio.2014.12.006>.
- Ortega-Beltran, A., Moral, J., Picot, A., Puckett, R.D., Cotty, P.J., Michailides, T.J., 2019. Atoxicogenic *Aspergillus flavus* isolates endemic to almond, fig, and pistachio orchards in California with potential to reduce aflatoxin contamination in these crops. *Plant Dis.* 103 (5), 905–912. <https://doi.org/10.1094/PDIS-08-18-1333-RE>.
- Padrón, H.Y.M., Delgado, S.H., Méndez, C.A.R., Carrillo, G.V., 2013. El género *Aspergillus* y sus micotoxinas en maíz en México: Problemática y perspectivas. *Revista Mexicana De Fitopatología* 31 (2), 126–146. <https://www.redalyc.org/articulo.oa?id=61231509005>.
- R Core Team. (2023). R: The R project for statistical computing. <https://www.r-project.org/> (last accessed 03 December 2024).
- Reid, L.M., Mather, D.E., Bolton, A.T., Hamilton, R.I., 1994. Evidence for a gene for silk resistance to *Fusarium graminearum* Schw. ear rot of maize. *J. Hered.* 85 (2), 118–121. <https://doi.org/10.1093/oxfordjournals.jhered.a111408>.
- Reveal Q+ MAX. (2023). Reveal Q+ MAX for aflatoxin | Mycotoxin testing. https://www.neogen.com/categories/mycotoxins/reveal-q-plus-max-aflatoxin/?utm_medium=SocialShare (last accessed 03 December 2024).
- Rodríguez-Del Bosque, L.A., Reyes-Méndez, C., Acosta-Núñez, S., Girón-Calderón, R., Garza-Cano, I., García-Villanueva, R., 1995. Control de aflatoxinas en maíz en Tamaulipas. Río Bravo, Tamaulipas México: instituto nacional de investigaciones agrícolas y pecuarias. *Folleto Técnico* 17, 20.
- Sarrocchio, S., Mauro, A., Battilani, P., 2019. Use of competitive filamentous fungi as an alternative approach for mycotoxin risk reduction in staple cereals: State of art and future perspectives. *Toxins* 11 (12), 701. <https://doi.org/10.3390/toxins11120701>.
- Savary, S., Nelson, A., Sparks, A.H., Willocquet, L., Duveiller, E., Mahuku, G., Forbes, G., Garrett, K.A., Hodson, D., Padgham, J., Pande, S., Sharma, M., Yuen, J., Djurle, A., 2011. International agricultural research tackling the effects of global and climate changes on plant diseases in the developing world. *Plant Dis.* 95 (10), 1204–1216. <https://doi.org/10.1094/PDIS-04-11-0316>.
- Sharma, M., Márquez, C., 2001. Determination of aflatoxins in domestic pet foods (dog and cat) using immunoaffinity column and HPLC. *Anim. Feed Sci. Technol.* 93 (1), 109–114. [https://doi.org/10.1016/S0377-8401\(01\)00274-7](https://doi.org/10.1016/S0377-8401(01)00274-7).
- Singh, N.A., Jyoti, Jain, V., 2023. Aflatoxins in food and feed: Occurrence, detection, and mitigating strategies. In: Kumar, P., Kamle, M.D., Mahato, D.K. (Eds.), *Mycotoxins in Food and Feed*. CRC Press.
- Soni, P., Gangurde, S.S., Ortega-Beltran, A., Kumar, R., Parmar, S., Sudini, H.K., Lei, Y., Ni, X., Huai, D., Fountain, J.C., Njoroge, S., Mahuku, G., Radhakrishnan, T., Zhuang, W., Guo, B., Liao, B., Singam, P., Pandey, M.K., Bandyopadhyay, R., Varshney, R.K., 2020. Functional biology and molecular mechanisms of host-pathogen interactions for aflatoxin contamination in groundnut (*Arachis hypogaea* L.) and maize (*Zea mays* L.). *Front. Microbiol.* 11, 227. <https://doi.org/10.3389/fmicb.2020.00227>.
- Sserumaga, J.P., Ortega-Beltran, A., Wagacha, J.M., Mutegi, C.K., Bandyopadhyay, R., 2020. Aflatoxin-producing fungi associated with pre-harvest maize contamination in Uganda. *Int. J. Food Microbiol.* 313, 108376. <https://doi.org/10.1016/j.ijfoodmicro.2019.108376>.
- Stutt, R.O.J.H., Castle, M.D., Markwell, P., Baker, R., Gilligan, C.A., 2023. An integrated model for pre- and post-harvest aflatoxin contamination in maize. *Npj Science of Food* 7 (1), 60. <https://doi.org/10.1038/s41538-023-00238-7>.
- Sweany, R.R., DeRobertis, C.D., Kaller, M.D., Damann Jr, K.E., 2022. Intraspecific growth and aflatoxin inhibition responses to atoxigenic *Aspergillus flavus*: evidence of secreted, inhibitory substances in biocontrol. *Phytopathology* 112 (10), 2084–2098. <https://doi.org/10.1094/PHYTO-01-21-0022-R>.
- Warburton, M.L., Brooks, T.D., Windham, G.L., Paul Williams, W., 2011. Identification of novel QTL contributing resistance to aflatoxin accumulation in maize. *Mol. Breed.* 27 (4), 491–499. <https://doi.org/10.1007/s11032-010-9446-9>.
- Weather-Spark. (2023). The weather year round anywhere on earth. <https://weatherspark.com/> (last accessed 03 December 2024).
- Womack, E.D., Williams, W.P., Windham, G.L., Xu, W., 2020. Mapping quantitative trait loci associated with resistance to aflatoxin accumulation in maize inbred Mp719. *Front. Microbiol.* 11, 45. <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00045>.
- Xing, F., Wang, L., Liu, X., Selvaraj, J.N., Wang, Y., Zhao, Y., Liu, Y., 2017. Aflatoxin B1 inhibition in *Aspergillus flavus* by *Aspergillus niger* through down-regulating expression of major biosynthetic genes and AFB1 degradation by atoxigenic *A. flavus*. *Int. J. Food Microbiol.* 256, 1–10. <https://doi.org/10.1016/j.ijfoodmicro.2017.05.013>.
- Xu, F., Baker, R.C., Whitaker, T.B., Luo, H., Zhao, Y., Stevenson, A., Boesch, C.J., Zhang, G., 2022. Review of good agricultural practices for smallholder maize farmers to minimise aflatoxin contamination. *World Mycotoxin J.* 15 (2), 171–186. <https://doi.org/10.3920/WMJ2021.2685>.
- Yin, G., Hua, S.S.T., Pennerman, K.K., Yu, J., Bu, L., Sayre, R.T., Bennett, J.W., 2018. Genome sequence and comparative analyses of atoxigenic *Aspergillus flavus* WRR1 1519. *Mycologia* 110 (3), 482–493. <https://doi.org/10.1080/00275514.2018.1468201>.
- Yu, J., 2012. Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination. *Toxins* 4 (11), 1024–1057. <https://doi.org/10.3390/toxins4111024>.
- Zhang, W., Dou, J., Wu, Z., Li, Q., Wang, S., Xu, H., Wu, W., Sun, C., 2022. Application of non-aflatoxigenic *Aspergillus flavus* for the biological control of aflatoxin contamination in China. *Toxins* 14 (10), 681. <https://doi.org/10.3390/toxins14100681>.