International Journal of Food Microbiology 405 (2023) 110336

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Assessment of early harvest in the prevention of aflatoxins in peanuts during drought stress conditions

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ARTICLE INFO

Keywords:
Pre-harvest
Groundnut
Aspergillus section *Flavi*
Management

ABSTRACT

The present study aimed to evaluate the effectiveness of early harvest in preventing aflatoxins in peanuts under drought-stress conditions. A field experiment was conducted on the 2018–2019 and 2019–2020 growing seasons in a greenhouse with an irrigation system to induce three drought stress conditions: no stress, mild, and severe stress. In addition, three harvest dates were proposed: two weeks earlier, one week earlier, and ideal harvest time. The mean peanut yield was 2634 kg/ha, considering the two growing seasons, and the drought stress conditions and harvest dates did not influence significantly. The shelling percentage was significantly higher in samples harvested at ideal harvest (77.7 %) than two weeks earlier (76.2 %) and was not influenced by drought stress conditions. Although a low mean percentage of grains with insect damage was identified, this percentage was statistically higher under severe stress (0.4 %) compared to no-stress conditions (0.2 %). The soil contamination ranged from 2.52×10^3 to 1.64×10^4 CFU/g of *Aspergillus* section *Flavi*, and the drought stress resulted in significantly higher concentrations in mild and severe stressed samples. *A.* section *Flavi* was found to infect all the peanut kernel samples. The drought stress resulted in higher percentages of *A.* section *Flavi* infections in samples from mild and severe stress conditions. The harvest date did not influence the soil and peanut kernel occurrence of *A.* section *Flavi.* A total of 435 and 796 strains of *A.* section *Flavi* were isolated from soil and peanut kernels, respectively. The potential of aflatoxin production by soil isolates was 31, 44, and 25 % for aflatoxin non-producers, aflatoxin B producers, and aflatoxin B and G producers, respectively, while in peanut kernel isolates were 44, 44, and 12 %. Three different *A.* section *Flavi* species were identified from peanut kernels: *A. flavus, A. parasiticus,* and *A. pseudocaelatus.* The mean aflatoxin concentration in peanut kernels was 42, 316, and 695.5 μg/kg in samples under no stress, mild stress, and severe stress conditions, respectively. Considering the harvest time, the mean aflatoxin concentration was 9.9, 334.3, and 614.2 μg/kg in samples harvested two weeks earlier, one week earlier, and in ideal harvest, respectively. In conclusion, the early harvest proved to be a viable, cost-free alternative for controlling aflatoxin in the peanut pre-harvest, resulting in a safer product and a better quality for sale and economic gain.

1. Introduction

The occurrence of *Aspergillus* section *Flavi* strains in peanut (*Arachis hypogaea*) crop is well-known and yet a problem to be controlled that might result in serious public health and economic issues (Pitt et al., 2012). *Aspergillus flavus* and *Aspergillus parasiticus* are the main *A.* section *Flavi* species naturally found in peanuts as commensal contaminants (Taniwaki et al., 2018). These species can grow in peanut kernels and, under drought stress conditions combined with high temperatures, produce aflatoxins, which are secondary metabolites classified as carcinogenic to humans (IARC, 2002). Furthermore, the aflatoxin occurrence in peanut kernel can also result in economic losses related to

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https://doi.org/10.1016/j.ijfoodmicro.2023.110336
Received 18 February 2023; Received in revised form 14 June 2023; Accepted 20 July 2023
Available online 23 July 2023

reduced yield, border rejection for exported products (considering levels above the destination country limit), and analytical costs for aflatoxin monitoring, among others (Lamb and Sternitzke, 2001; Meneely et al., 2022, Wu et al., 2008).

The development and maturation of peanuts in the field occur below the ground (Bertioli et al., 2011; Tallury, 2017). Since soil is an extensive reservoir of aflatoxigenic fungi, including *A. flavus* and *A. parasiticus*, this peanut-growing condition exposes them to potential pre-harvest contamination and aflatoxin production (Horn, 2005). Another risk is post-harvest contamination during peanut storage. After drying, if peanuts are not stored in a controlled temperature and relative humidity environment, the fungus present in stored peanuts may have favorable conditions for aflatoxins production.

Several studies have listed preventive measures to reduce aflatoxin occurrence in the peanut supply chain from pre- to post-harvest stages (Dorner, 2008; Guo et al., 2009; Rachaputi et al., 2002; Torres et al., 2014). Biological control (Moore, 2022; Peles et al., 2021), genetic resistance (Yu et al., 2020), crop rotation, and irrigation to prevent drought stress (Lamb et al., 2010) are the main strategies discussed for aflatoxin prevention in pre-harvest peanut.

Despite the proposals mentioned above for aflatoxins pre-harvest control, some countries still face difficulties implementing these measures. In Brazil, for example, peanuts are planted in crop rotation with sugarcane. Cultivars with better-fit planting conditions in a significant producing state (São Paulo) are not resistant to drought stress or *Aspergillus* sp. infection. In addition, implementing biocontrol and irrigation systems is not economically attractive for producers.

Considering cost-effective management strategies to prevent aflatoxin in pre-harvest peanuts, changing the sowing date (Craufurd et al., 2006), and early harvesting in drought stress conditions would be useful and economically viable alternatives (Rachaputi et al., 2002). So, the present study aimed to: (i) analyze whether drought stress conditions influence the profile of *A.* section *Flavi* strains isolated in soil and peanut kernels, (ii) assess if early harvesting reduces the risk of peanut infection by *Aspergillus* section *Flavi*, (iii) evaluate if an earlier harvest when in drought stress conditions would prevent aflatoxin occurrence during peanut pre-harvest, (iv) evaluate peanut yield and characteristics under different drought stress conditions and harvest times.

2. Material and methods

2.1. Field experiment

A field experiment was carried out in a greenhouse with a drip irrigation system in a peanut-producing region (21°42′29.9″S 48°12′11.8″ W) in São Paulo state, Brazil. A high oleic peanut cultivar (IAC OL3) was grown for two consecutive crop seasons, from Dec 2018 to April 2019 and from Oct 2019 to Feb 2020. The plantation was split into three parts that received different irrigation treatments. Each part size consisted of 14 × 12 m, with 13 rows of 14 m each and a spacing of 90 cm between rows. The two border edge rows were discarded to avoid border effects.

During the vegetative growth, flowering, and beginning of peg formation, all three parts were irrigated equally with the same amount of water. Approximately 60 days after sowing, each part was irrigated at different intervals (Craufurd et al., 2006).: (i) every seven days, simulating a condition with no drought stress (no stress), (ii) every 14 days, simulating a mild stress condition (mild stress), (iii) every 21 days, simulating a severe stress condition (severe stress). The soil moisture and temperature were monitored using two sensors (temperature smart sensor S-TMB-M002 and soil moisture smart sensor S-SMC-M005, Onset, Bourn, USA) placed at different locations in the plantation. The moisture and temperature data were recorded every 30 min in a data logger. In each of the three drought stress conditions, three rows (each one corresponding to a peanut sample) were harvested on three different dates: two weeks before the ideal harvest, one week before, and at the ideal harvest time, resulting in 27 samples from each planting year (a total of 54 samples). The ideal harvest date was determined by monitoring the crop development, collecting aleatory plants from each drought stress condition, and counting the percentage of mature seeds. Soil samples were collected from each row. Peanut samples were harvested, sun-dried to <8 % kernel moisture, and kept in the shell until analysis.

2.2. Water activity and kernel moisture

Water activity analysis was performed using the Aqualab, model 3TE (Decagon, USA) at 25 °C ± 1 °C. The kernel moisture was measured by gravimetry according to the AOAC methodology (2009).

2.3. Peanut yield, shelling percentage, and insect damage

The peanut yield was calculated considering the row extension of 12 m in triplicate (disregarding 1 m from each edge from the 14 m line) and multiplying by 0.9 m (spacing between rows), totaling 10.8 m^2 of area. Then, the dry peanut pod was weighed, corresponding to this area, and calculated in kg/ha.

The shelling percentage was calculated using 500 g of random peanut pod samples. Then, the ratio of the kernel to pod weight was determined and expressed as a percentage. Finally, 500 g of peanut kernel was used to evaluate the percentage of insect damage.

2.4. Aspergillus section Flavi isolation and morphological identification

The soil samples (54) were analyzed with the plate dilution technique, according to Pitt and Hocking (2009). First, the soil (25 g) was weighed and added to 225 mL of aqueous 0.1 % peptone. Then, serial dilutions were carried out, and 0.1 mL were pipetted onto the DG18 medium and spread over the surface. Finally, the plates were incubated for five days at 25 °C, and the results were expressed as colony-forming units per gram (CFU/g).

The mycological analysis of the peanut kernels was performed using the direct plating method, according to Pitt and Hocking (2009). First, the peanut kernel samples were superficially disinfected in a 0.4 % hypochlorite solution for 2 min under agitation. Next, 50 grains were directly plated and distributed in 10 plates (5 grains per plate) containing 18 % glycerol Dicloran agar (DG18). Finally, the plates were incubated at 25 °C for five days, and the results were expressed as a percentage of infection (Pitt & Hocking, 2009).

For the identification of the isolates, the strains suspected of belonging to the *Aspergillus* section *Flavi* were inoculated on the Czapek yeast extract (CYA) medium and incubated at temperatures of 25 °C and 37 °C for seven days and morphologically identified by the classification key Pitt and Hocking (2009) and Samson et al. (2010).

2.5. Molecular analysis

A total of 196 isolates, putatively characterized as belonging to *A.* section *Flavi*, were selected based on their phenotypic characteristics (aflatoxin production and morphology) for molecular identification.

For genomic DNA extraction, the strains were purified through monosporic cultivation and later cultivated in liquid yeast extract sucrose at 25 °C for 3 days until the formation of a mycelial film. Then, the mycelia were removed from the culture medium and macerated with liquid nitrogen. Finally, genomic DNA was extracted using the DNA Purification Kit (Mebep Bioscience, Shenzhen, China) according to the manufacturer's protocol.

For species-level identification, the amplification of part of the gene encoding calmodulin (*CaM*) was performed using primer-pair CMD5 and CMD6 (Hong et al., 2006). The amplification conditions were the same as Silva et al. (2020). The amplification products were purified using ExoSAP-IT™ PCR Product Cleanup reagent (Thermo Fisher Scientific, USA). Finally, the amplicons were sequenced in both directions using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems,

USA) in a SeqStudio Genetic Analyzer (Applied Biosystems, USA).

The sequences obtained were aligned with sequences from all *A.* section *Flavi* type strains available in the NCBI database (http://www.ncbi.nlm.nih.gov) using ClustalW (Thompson et al., 1994). The maximum-likelihood trees (ML) were inferred using the Tamura-Nei model (Tamura and Nei, 1993) with invariant sites plus gamma distribution (I + G). To determine each clade support, a bootstrap analysis was performed with 1000 replicates using MEGA 7.0 software (Kumar et al., 2016).

2.6. Potential of aflatoxin production by isolates

All the *A.* section *Flavi* strains were purified and inoculated on Yeast Extract and Sucrose agar (YESA) and incubated at 25 °C for seven days. Then, the agar plug technique associated with thin layer chromatography (TLC) was applied, according to Filtenborg et al. (1983). A plug was removed from the culture medium, and three drops of methanol: chloroform (1: 1) were added. The plugs were applied to the 500 μm thick TLC silica gel-G plates with the aflatoxin standard. The mobile phase was toluene: ethyl acetate: 90 % formic acid: chloroform (7: 5: 2: 5 v / v / v / v). The plate was read in a UV chamber at 356 and 254 nm wavelengths. The retention factor and fluorescence were compared to aflatoxin standards B1, B2, G1, and G2 to assess their presence in the tested strains. Unclear results were confirmed qualitatively in high-performance liquid chromatography (HPLC). An *A.* section *Flavi* plug was removed and collected in a vial. Next, methanol (approximately 1 mL) was added, and the mixture was homogenized using a vortex. Finally, the solution was double filtred in a 0.22 μm Millex connected to a syringe and injected at HPLC as described in item 2.7.

2.7. Aflatoxins analysis in peanut kernel samples

The aflatoxins analysis peanut samples was carried out in all 54 (in triplicate) according to Stroka et al. (2000), with modifications according to Martins et al. (2017). Approximately 500 g of peanuts were ground, and 25 g of the milled sample was added to 2.5 g of NaCl and 100 mL of methanol: water (8: 2 v/v). This solution was homogenized in a horizontal shaker (New Brunswick Scientific Company, New Brunswick, NJ, USA) for 30 min. It was then doubly filtered on quantitative filter paper (Unifil, Brazil) and microfibre filters 1.5 μm 11 cm (Vicam, Milford, MA, USA), respectively. Next, 10 mL of the final filtrate was diluted in 60 mL of phosphate buffer solution (PBS pH 7). The total content was passed through an immunoaffinity column (Aflatest WB, Vicam), with a flow of 1–2 drops per second. Then, the column was washed with 30 mL of distilled water. Finally, the aflatoxins were eluted with 1250 μL of HPLC grade methanol and 1750 μL of ultra-pure water (MiliQ, Merck KGaA, Germany).

For aflatoxin quantification, a high-performance liquid chromatography (HPLC) (Model 1260 Infinity, Agilent, Santa Clara, CA, USA) was used, with a fluorescence detector (362 nm excitation and 455 nm emission). A C18 reverse-phase column (Zorbax Eclipse Plus 4.6 × 150 mm 5 μm, Agilent, USA) was used, and the oven temperature was 40 °C. For the post-column derivatization of B1 and G1 aflatoxins, a Kobracell electrochemical reactor (R-Biopharm, Darmstadt, Germany) was connected to a current of 100 μA. The mobile phase was composed of water: acetonitrile: methanol (6: 2: 3, v / v / v), added with 119 mg of KBr and 350 μL of nitric acid 4 M per liter, in an isocratic system with a continuous flow of 1 mL/min., and 20 μL as the volume of injection. The detection and quantification limits for aflatoxin B1, B2, G1, and G2 were 0.02, 0.01, 0.01, and 0.01 μg/kg and 0.06, 0.05, 0.04, and 0.02 μg/kg, respectively.

2.8. Statistical analysis

Data were analyzed using the SAS software (SAS Institute Inc. The SAS System, release 9.4. SAS Institute Inc., Cary:NC:USA, 2012). A generalized linear mixed model (GLMM) was used for a completely randomized experiment with the irrigation effect and repeated measures for the impact of harvesting. The two seasons were considered as a random effect. The variables that showed a significant difference were analyzed using the Tukey test. The chi-squared test was used to evaluate the frequency of strains with different aflatoxin production potentials (non-producers, aflatoxin B producers, and aflatoxin B and G producers) under different drought stress conditions, using the software R (version 4.2.0).

3. Results

3.1. Climatic conditions during the growing season (2018–19 and 2019–20)

The soil moisture (Table 1) and temperature (Table 2) were monitored during the two growing seasons considering each reproductive growth stage according to Boote (1982), from blooming (R1) to peanut maturity (R8). Soil moisture values from 0 to 0.1m^3/m^3 indicated over-dry to dry soil, respectively, while values of 0.3 m^3/m^3 indicated wet or saturated soil.

The soil moisture values ranged from 0.22 m^3/m^3 in no-stress conditions to 0.09 m^3/m^3 in severe stress conditions. Soil moisture values were higher than 0.1 m^3/m^3 during the first 60 days after sowing for the three irrigation conditions. After that, the soil moisture for no-stress conditions ranged from 0.22 to 0.13 m^3/m^3 from the beginning of seed (R5) to harvest maturity (R8), respectively. For mild stress conditions, the moisture continuously dropped from 0.19 m^3/m^3 (R5) to 0.10 m^3/m^3 (R8). The severe stress part showed values varying from 0.12 to 0.09 m^3/m^3, from R5 to R8, respectively, indicating the most dried soil condition.

The mean soil temperature was higher at the beginning of the growing season, reaching 29.4 °C, and after the 60 first days after sowing, the mean temperature went down to 25–26 °C. The severe stress condition presented the highest temperature range (from 37.9 to 21.7 °C) and the highest temperature values, with a maximum mean temperature above 30 °C in all stages of the growing season.

3.2. Peanut yield, shelling percentage, and insect damage

Table 3 shows the peanut yield (kg/ha), shelling percentage, and insect damage (%) in peanut samples from the two growing seasons (2018–19 and 2019–20).

The peanut yield (kg/ha) ranged from 2362 kg/ha (mild stress, two weeks earlier) to 3000 kg/ha (no stress, ideal harvest). The drought stress condition and harvest dates proposed in this study did not significantly influence peanut yield (p value = 0.1354 and 0.067, respectively).

Table 1
Mean soil moisture (m^3/m^3) from the two growing seasons (2018–19 and 2019–20) in each reproductive stage of peanut growth.

Reproductive stage*	DAS	Soil moisture (m^3/m^3) - mean ± sd		
		No stress	Mild stress	Severe stress
Beginning Bloom (R1)	0–31	0.17 ± 0.06	0.11 ± 0.02	0.09 ± 0.03
Beginning Peg (R2)	32–42	0.21 ± 0.04	0.16 ± 0.05	0.14 ± 0.03
Beginning Pod (R3)	43–51	0.2 ± 0.05	0.18 ± 0.04	0.15 ± 0.04
Full Pod (R4)	52–60	0.21 ± 0.05	0.18 ± 0.03	0.15 ± 0.02
Beginning seed (R5)	61–62	0.22 ± 0.05	0.19 ± 0.03	0.15 ± 0.03
Full seed (R6)	63–74	0.19 ± 0.05	0.14 ± 0.04	0.12 ± 0.03
Beginning maturity (R7)	75–93	0.17 ± 0.04	0.12 ± 0.04	0.11 ± 0.03
Harvest maturity (R8)	94–125	0.13 ± 0.03	0.1 ± 0.04	0.09 ± 0.03

DAS = days after sowing; * According to Boote, 1982; ** Soil moisture was presented as volumetric water content. Values range from 0 to 0.5m^3/m^3, in which the value of 0 to 0.1 m^3/m^3 indicates an over-dry to dry soil, respectively, and values of 0.3 m^3/m^3 indicate a wet or saturated soil.

Table 2
Soil temperature (°C) from the two growing seasons (2018–19 and 2019–20) in each reproductive stage of peanut growth.

Reproductive stage	DAS	No stress			Mild stress			Severe stress		
		Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Beginning Bloom (R1)	0–31	28.2 ± 1.4	32.4 ± 2.4	24.9 ± 1.9	29.4 ± 1.5	33.8 ± 2.7	25.4 ± 0.9	28.3 ± 1.7	37.9 ± 3.6	21.7 ± 3.5
Beginning Peg (R2)	32–42	28.5 ± 2.6	31.8 ± 3.8	26.2 ± 1.3	29.0 ± 1.9	31.9 ± 3.2	25.6 ± 0.8	27.6 ± 2.5	32.0 ± 3.0	23.6 ± 4.1
Beginning Pod (R3)	43–51	27.8 ± 2.1	29.5 ± 2.5	24.2 ± 1.0	28.4 ± 2.0	30.8 ± 2.8	24.6 ± 0.8	27.4 ± 1.4	34.4 ± 2.8	22.8 ± 5.2
Full Pod (R4)	52–60	26.4 ± 1.3	27.8 ± 1.4	24.1 ± 0.9	26.9 ± 1.3	28.4 ± 1.7	24.6 ± 0.7	25.9 ± 1.0	32.6 ± 1.5	22.8 ± 3.8
Beginning seed (R5)	61–62	26.0 ± 0.5	27.2 ± 0.9	24.0 ± 0.9	26.4 ± 0.6	28.0 ± 1.2	25.0 ± 0.1	25.5 ± 0.5	31.0 ± 0.6	23.0 ± 2.6
Full seed (R6)	63–74	25.6 ± 0.7	27.2 ± 1.2	23.8 ± 0.8	26.0 ± 0.7	27.5 ± 1.1	24.3 ± 0.4	25.2 ± 0.6	32.6 ± 3.3	22.6 ± 2.6
Beginning maturity (R7)	75–93	25.5 ± 0.8	27.0 ± 1.1	23.5 ± 0.7	26.2 ± 0.9	28.3 ± 1.4	23.1 ± 5.5	25.6 ± 0.9	34.4 ± 4.1	23.0 ± 3.1
Harvest maturity (R8)	94–125	25.2 ± 0.6	27.2 ± 0.6	23.5 ± 1.0	26.9 ± 0.7	29.8 ± 1.4	24.9 ± 0.9	25.7 ± 1.1	32.0 ± 4.2	23.1 ± 2.8

DAS = days after sowing; * According to Boote, 1982.

Table 3
Mean ± sd of peanut yield (kg/ha), shelling percentage, and insect damages (%) from the two growing seasons (2018–19 and 2019–20) at different drought stress conditions and harvest dates.

Harvest	Peanut Yield (kg/ha)			Shelling percentage (%)			Insect damage (%)		
	No stress	Mild stress	Severe stress	No stress	Mild stress	Severe stress	No stress	Mild stress	Severe stress
Two weeks earlier	2550 ± 654	2362 ± 1137	2580 ± 1179	75.8 ± 2.7	75.8 ± 2	77.1 ± 1.1	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
One week earlier	2735 ± 737	2653 ± 1088	2557 ± 1119	75.1 ± 2.7	76.8 ± 1.8	78.2 ± 1.5	0.2 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
ideal harvest	3000 ± 718	2657 ± 1106	2614 ± 1186	78.2 ± 1.9	78.2 ± 2.6	77.4 ± 2.5	0 ± 0	0.2 ± 0.2	0.3 ± 0.4

Means with different letters indicate significant differences by Tukey's multiple tests ($P < 0.05$).

The harvest date significantly influenced the shelling percentage ($p = 0.0181$). Samples harvested two weeks earlier presented an average of 76.2 % shelling percentage, while samples harvested one week earlier presented 76.7 % and, at ideal harvest, 77.7 %. There was no significant difference in the shelling percentage on the drought stress conditions proposed in the present study ($p = 0.0510$).

The average percentage of peanut samples with insect damage among different drought stress conditions was significant ($p = 0.0389$) and ranged from 0.2 % for samples under no drought stress to 0.4 % for severe stress conditions. Different harvest dates did not influence insect damage ($p = 0.1331$).

3.3. Isolation and morphological and molecular identification

The mean peanut kernel water activity (a_w) and peanut kernel moisture from the two consecutive growing seasons (2018–2019 and 2019–2020) were presented in Table 4. The water activity ranged from 0.519 to 0.612, while the moisture ranged from 5.5 to 6.7 %. Therefore, the kernel moisture followed national standards (below 8 %) (Brasil, 2016), and the low water activity indicated a safe condition to prevent fungal growth during storage (FDA, 2014).

Fig. 1 shows the mean *A.* section *Flavi* soil contamination (log CFU/g). The soil contamination ranged from 2.52×10^3 CFU/g in no-stress samples at the ideal harvest to 1.64×10^4 CFU/g in mild-stress samples at the ideal harvest. The soil contamination was statistically higher ($p < 0.001$) in samples with mild and severe stress than in the samples with no stress, and the harvest date was not a variable that contributed significantly ($p = 0.8153$) to the soil contamination.

The mean percentage (%) of total mold infection on peanut kernel samples ranged from 50 to 98 %. *A.* section *Flavi* was present in all samples (Fig. 2). The *A.* section *Flavi* % of infection (AFi) in samples

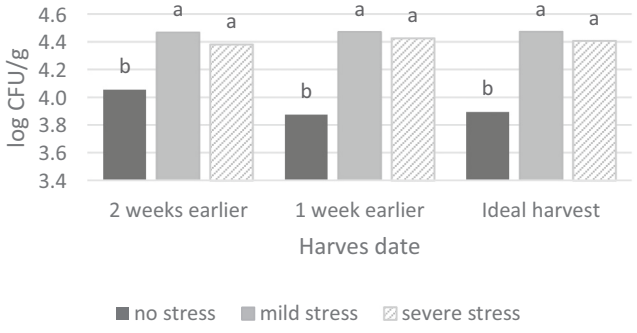

Fig. 1. Mean soil contamination (log CFU/g) of *A.* section *Flavi* from the two growing seasons (2018–19 and 2019–20) at different drought stress conditions and harvest dates. Means with different letters indicate significant differences by Tukey's multiple tests ($P < 0.05$).

under mild and severe drought stress was statistically higher ($p < 0.0001$) than in the no-stress condition. However, the difference among the harvest dates was not statistically significant ($p = 0.1219$). Under no-stress conditions, the AFi was 32, 21, and 32 % when harvested two weeks earlier, one week earlier, and ideal harvest, respectively, while for mild and severe stress ranged from 50, 70, 73 %, and 52, 83, and 82 %, respectively.

Fig. 3 shows the ML tree based on the *CaM* locus showing the relationships between *A.* section *Flavi* species and isolates of peanut kernel samples. Among the 196 isolates selected for molecular analysis, three species were identified: *Aspergillus flavus* (118 isolates), *Aspergillus parasiticus* (75), and *Aspergillus pseudocaelatus* (3).

Table 4
Mean ± sd water activity (A_w) and kernel moisture from the two growing seasons (2018–19 and 2019–20).

Harvest	No stress		Mild stress		Severe stress	
	Aw	Moisture (%)	Aw	Moisture (%)	Aw	Moisture (%)
Two weeks earlier	0.595 ± 0.03	6.7 ± 0.4	0.553 ± 0.05	5.6 ± 0.5	0.582 ± 0.03	5.6 ± 0.6
One week earlier	0.519 ± 0.11	5.5 ± 1.0	0.597 ± 0.04	6.0 ± 0.3	0.584 ± 0.09	5.7 ± 1.5
ideal harvest	0.612 ± 0.02	6.5 ± 0.4	0.596 ± 0.06	6.0 ± 0.9	0.559 ± 0.05	5.6 ± 0.8

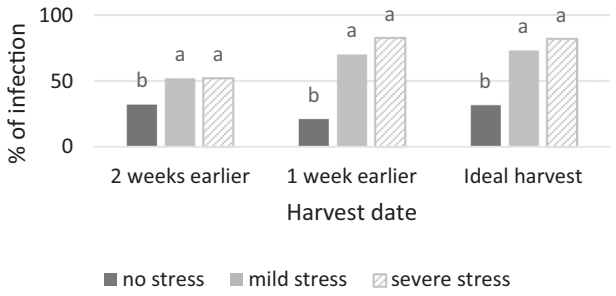

Fig. 2. Mean peanut kernel percentage of *A.* section *Flavi* infection from the two growing seasons (2018–19 and 2019–20) at different drought stress conditions and harvest dates. Means with different letters indicate significant differences by Tukey's multiple tests ($P < 0.05$).

3.4. Potential of aflatoxin production by isolates

All isolates were tested for their aflatoxin production potential. The percentage of isolates non-producers, aflatoxin B, and aflatoxin B and G producers on soil and peanut kernels are shown in Fig. 4. The chi-squared test showed that the frequency of *A.* section *Flavi* strains with different aflatoxin production potentials varied significantly in soil ($X^2 = 21.2$, $df = 4$, $p\text{-value} = 0{,}0002$) and peanut kernel ($X^2 = 18.7$, $df = 4$, $p\text{-value} = 0{,}0009$), indicating an influence of the studied conditions.

A total of 435 and 796 A. section *Flavi* strains were isolated from soil and peanut kernels samples, respectively, during the two growing seasons. From soil isolates, 31 % (136) were aflatoxin non-producers, 44 % (189) were aflatoxin B producers, and 25 % (110) were aflatoxin B and G producers, while for peanut kernel isolates, the distribution was 44 % (352), 44 % (350), and 12 % (94), respectively.

The aflatoxin production by soil isolates showed a different profile from the peanut kernel isolates. Under no stress conditions, the soil isolates' aflatoxin B producers stood out with the highest percentage in the earlier harvest. In contrast, aflatoxin non-producers and aflatoxin B and G producers presented similar percentages. However, in the soil under severe stress conditions, the distribution of the isolates was more homogeneous, with approximately the same percentage of non-producers (26 %), aflatoxin B (36 %), and aflatoxin B and G producers (38 %). On the other hand, the isolates from the peanut kernel samples had a higher percentage of non-producers (33 %) and aflatoxin B producers (53 %) and a low percentage of aflatoxin B and G producers (14 %).

3.5. Aflatoxins in peanut kernels

Drought stress ($p = 0.022$) and harvest date ($p = 0.0002$) influenced the aflatoxin concentration in peanut samples. The combination of these variables (drought stress and harvest date) was not significant ($p = 0.0626$). On average, aflatoxin concentration on samples under no-stress conditions was 14.3, 10.8, and 100.9 μg/kg from samples harvested two weeks earlier, one week earlier, and ideal harvest, respectively. Under mild stress conditions, aflatoxin concentrations were 11.8, 182.5, and 754.8 μg/kg from 2 weeks, one week earlier, and ideal harvest, respectively. Finally, the severe stress resulted in aflatoxin concentrations of 3.6, 809.6, and 959.7 μg/kg, respectively. The mean aflatoxin concentration in drought stress and harvest dates conditions were illustrated in Fig. 5 (A and B, respectively). The concentration was higher in samples harvested at ideal harvest under mild and severe stress. The results showed that under drought stress conditions, samples harvested two weeks earlier and one week earlier presented 62 and 2 times lower aflatoxin contamination in peanut kernels, respectively.

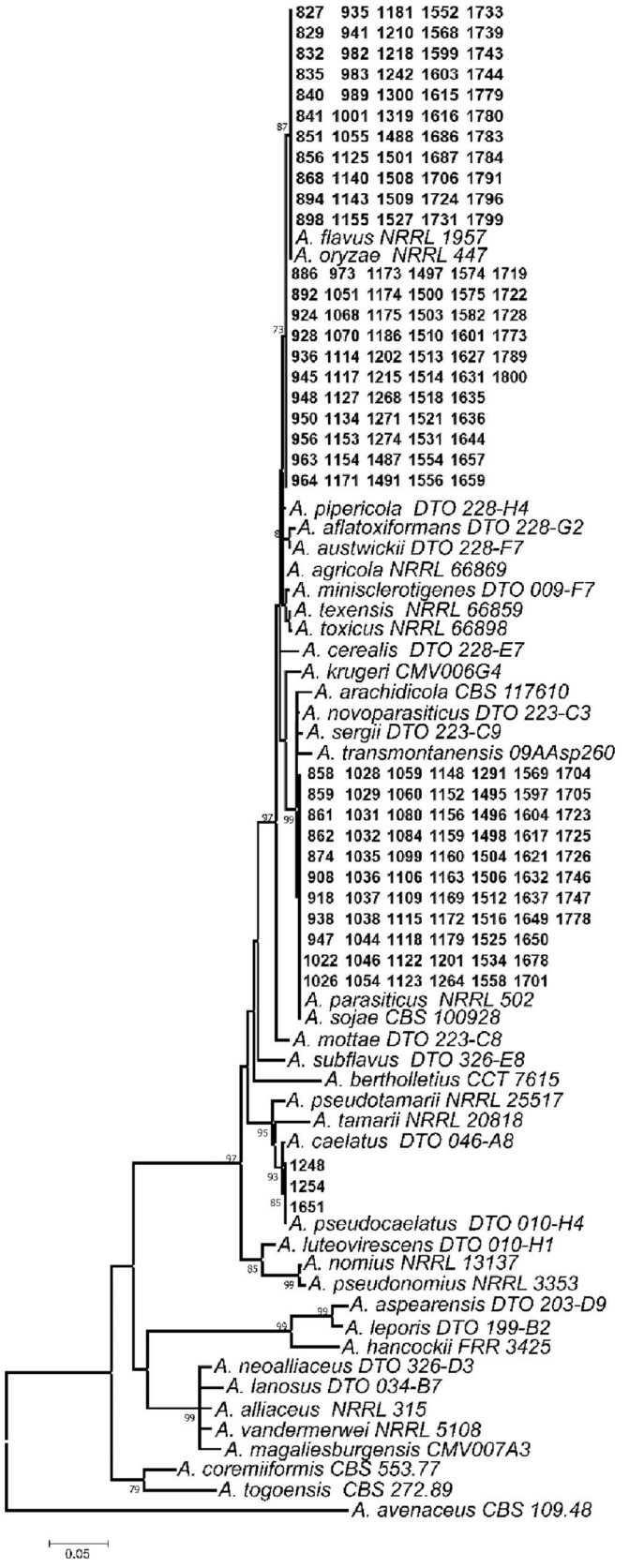

Fig. 3. Maximum likelihood tree based on the CaM gene showing the relationships between *Aspergillus* section *Flavi* species and isolates of peanut kernel samples.

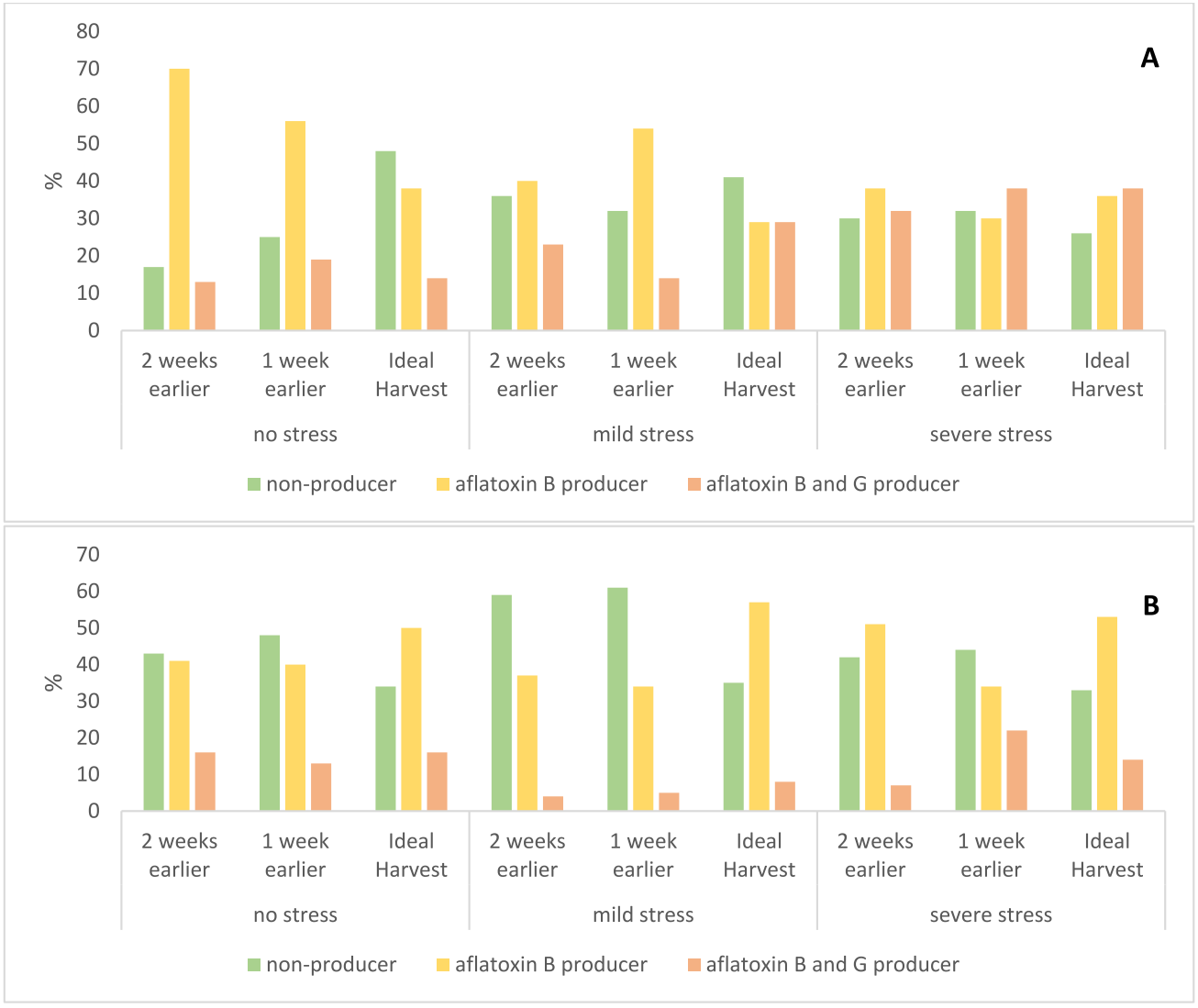

Fig. 4. Percentage of non-producers, aflatoxin B and aflatoxin B and G producers isolated from soil (A) and peanut kernels (B) from the two growing seasons (2018–19 and 2019–20).

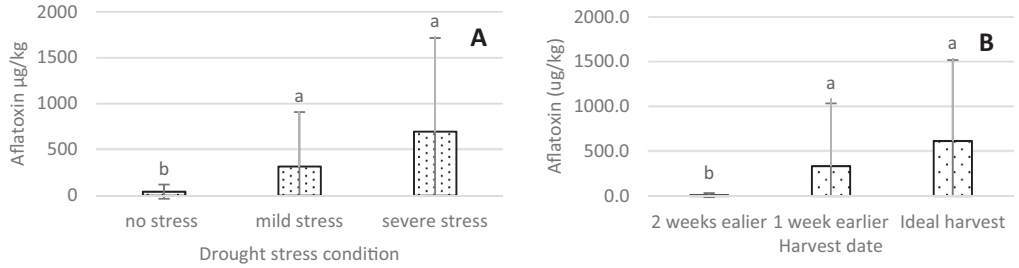

Fig. 5. Mean aflatoxin concentration in different drought stress conditions (A) and harvest dates (B) from the two growing seasons (2018–19 and 2019–20). Means with different letters indicate significant differences by Tukey's multiple tests ($P < 0.05$).

4. Discussion

The results from climatic conditions showed that the proposed conditions (different harvest dates and drought stress conditions) could induce different drought stress during the peanut growing season. The severe stress conditions presented the lowest soil moisture and the highest soil temperature, in agreement with Jeyaramraja et al. (2018), that the soil temperature increases during drought stress conditions. According to Cole et al. (1985), the upper soil temperature limit for aflatoxin production is from 29.6 to 31.3 °C, while the lower temperature is between 25.7 and 26.3 °C. So, even presenting a temperature decrease by the R6 stage (whole seed formed), the mean values were within the ideal temperature for aflatoxin production.

The mean peanut yield in each study condition was lower than the yield found by De Godoy et al. (2014) for the IAC OL3 cultivar, which ranged from 3240 to 5823 kg/ha in peanuts grown in different

experimental fields and crop years. The influence of drought stress on peanut yield varies depending on the maturation stage at which the plant undergoes stress. For example, during the reproductive stages between pegging and pod development, drought stress causes a more significant reduction in peanut yield compared to stages from pod development to maturation (Reddy et al., 2003). Therefore, the lack of a statistically significant decrease in peanut yield in samples with drought stress (mild and severe) might be related to the period that the drought stress was imposed.

The shelling percentages results were within the range of Canavar and Kaynak (2008), from 50 to 83 % in two seasons and different cultivars. Although the drought stress conditions of the present study did not influence the shelling percentage, a reduced shelling percentage in drought stress during kernel or seed development has been reported due to the reduced pod and seed weight (Reddy et al., 2003).

The increased insect damage percentage, *Aspergillus* section *Flavi* percentage of infection, and aflatoxins occurrence under drought stress seem to be mainly related to a decrease in the plant's defense system. Under normal conditions, the plant can produce phytoalexins (chemical compounds) in response to pathogen attacks. However, this production is reduced under drought stress (Pitt et al., 2013). In addition, there are biochemical changes in the stressed plant, such as the production of soluble carbohydrates and amino acids in the leaves, which are easily used by insects, increasing their infestation. Thus, it was expected that the most significant percentage of insect injury seeds occurred in the most stressed samples (Reddy et al., 2003). Therefore, insect damage in peanuts pod creates an entry that facilitates fungal contamination and possibly aflatoxin production in peanut kernels.

The molecular analysis identified three species of *Aspergillus* section *Flavi* in peanut kernels: *A. flavus, A. parasiticus,* and *A. pseudocaelatus*. The first two species are known and described with great frequency in peanut crops, with *Aspergillus flavus* being reported more frequently than *Aspergillus parasiticus* (Barros et al., 2006; Horn, 2007; Pitt and Hocking., 2009; Taniwaki et al., 2018; Vaamonde et al., 2003). Among the 94 isolates with the potential for aflatoxin B and G production, 75 (80 %) were molecularly identified as *Aspergillus parasiticus*, and only 3 (3 %) were identified as *Aspergillus pseudocaelatus*. Varga et al. (2011) isolated and identified this species from *Arachis burkartii* leaf in Argentina. Since then, this species has been reported in other commodities, such as Brazil nut shells (Taniwaki et al., 2017), rice (Katsurayama et al., 2018), Brazilian dry beans (Santos-Ciscon et al., 2019) and medicinal plants (Zohair et al., 2018). In addition, *A. pseudocaelatus* was reported in peanut samples from Brazil by Frisvad et al. (2019).

Aflatoxin occurrence in peanut kernel samples under drought stress conditions and harvested at the ideal time showed the highest mean values. In contrast, earlier harvest in the same stress condition presented aflatoxin levels within limits acceptable by Brazilian legislation (20 μg/kg) (Brasil, 2022). These results are promising for aflatoxin control during peanut pre-harvest, considering that no additional care or procedure during the growing season is needed. The main challenge the producer may face is the knowledge that the plantation is under drought stress and at risk of aflatoxin production. Some predictive models have been created to identify plant stress conditions by monitoring climatic conditions such as soil/ambient temperature and rainfall. For instance, the Afloman (Chauhan et al., 2010), developed in Australia, has been used as a decision-support tool to monitor pre-harvest aflatoxin risk in peanuts, and DSSAT-CROPGRO-Peanut (Boote et al., 1998) developed in the United States was successfully used to predict aflatoxin occurrence in Niger by Craufurd et al. (2006).

Considering the Brazilian scenario between peanut cooperatives (non-profit organizations that act as processors and wholesales) and producers, the present study's results would benefit mainly cooperatives. Cooperatives usually buy peanuts from the associated producers, and there is no difference in price for peanuts, regardless of aflatoxin contamination. Thus, peanut producers are not motivated to deliver a peanut with the highest quality since the price would not change.

Some countries, such as Australia, have used pricing penalties for producers who sell peanuts with aflatoxin contamination (Rachaputi et al., 2002). On the one hand, these policies may induce the need for strategies to minimize aflatoxin contamination. But on the other hand, these price penalties may discourage peanut producers from continuing their production through economic losses. Therefore, a producer bonification policy would be a possible way for the cooperative to encourage constant monitoring by transferring the profit to the producer with the value obtained from selling higher-quality peanuts.

In conclusion, the earlier harvest under drought stress showed that the aflatoxin production was prevented even with more significant insect damage and soil and peanut contamination by *Aspergillus* section *Flavi*. In addition, in the conditions of the present study, the peanut yield in an earlier harvest was similar to the ideal harvest. Therefore, the simple and cost-free proposal of an earlier harvest proved a viable alternative with practical potential for its application in the field.

Declaration of competing interest

Ligia Manoel Martins reports financial support was provided by Coordination of Higher Education Personnel Improvement. Marta Hiromi Taniwaki reports financial support was provided by State of Sao Paulo Research Foundation. Marta Hiromi Taniwaki reports financial support was provided by National Council for Scientific and Technological Development. Neura Bragagnolo reports financial support was provided by National Council for Scientific and Technological Development.

Data availability

Data will be made available on request.

Acknowledgments

The authors also acknowledge CAP Agroindustrial and Coplana for all the support with peanut shelling percentage and insect damage analysis.

Funding

The authors acknowledge the financial support of "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP) (Grant # 2017/00824-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant # 303732/2018-0 and 304131/2019-9) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Finance code 001).

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