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Short Notes

“AFLA-peanut”, a mechanistic prototype model to predict aflatoxin B1 contamination

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Summary. Italian production of peanuts has recently increased. Aflatoxin B1 (AFB1) contamination of peanuts is currently not in Italy, but changing climatic conditions of the Mediterranean region may increase risks posed by this mycotoxin. A mechanistic weather-driven prototype model to predict AFB1 contamination in peanuts was developed by adapting the mechanistic AFLA-maize model for the *Aspergillus flavus*-peanut pathosystem. The peanut growth stages were examined to develop a phenology model based on growing degree days (GDD), which was linked to an *A. flavus* infection cycle model, and exploited to develop the “AFLA-peanut” prototype model. Starting from sowing, 686 GDD were required to reach flowering (as the critical growth stage for *A. flavus* infection), and 1925 GDD were required to reach harvesting, in a short season peanut variety. Variability of the AFB1 index, across years and locations, highlighted the capacity of AFLA-peanuts to account for weather data inputs in predicting AFB1 contamination risks. Although model validation will be mandatory to assess AFLA-peanut performance, this study has provided the first evidence that the prototype model could become an important tool for aflatoxin risk management.

Keywords. *Aspergillus flavus*, model transfer, weather, phenology, mycotoxin, climate change.

INTRODUCTION

Peanut (*Arachis hypogaea* L., *Fabaceae*), also known as groundnut, is an annual herbaceous plant, which is native to Central and South America, and is characterized by fruit development beneath soil surfaces. Peanut crop production now occurs in many countries thanks to its beneficial nutritional properties (Mingrou *et al.*, 2022). Annual world peanut production has grown by over 54 million tons per year, with China as the main producer, followed by India, Nigeria, and the United States of America (FAOSTAT, 2024). In the Mediterranean region, peanut production is widespread, especially in Turkey and Egypt, and limited production occurs in Spain and Greece (Sannino *et al.*, 2020; Özkaya *et al.*, 2024). Italian peanut production is increasing, from 22 tons per year in 2017 to 712 tons per year in 2024 (Istat, 2024).

Aspergillus flavus infects and colonizes several crops, and among these, peanut is one of the most susceptible (Horn, 2005; Amaike and Keller, 2011; Payne, 2014). Aflatoxins (AFs) are toxic and cancerogenic substances. Consumption of food and feed contaminated by these compounds can cause several harmful effects, including genotoxicity, hepatotoxicity, carcinogenicity, and nephrotoxicity, and have mutagenic, teratogenic, cytotoxic, and immunosuppressive effects (Shephard, 2008; Aristil *et al.*, 2020, Singh *et al.*, 2021). Contamination can occur or increase throughout the peanut supply chain, from the field, during crop growth, natural drying after digging, harvesting, storage and product delivery, and to eventual processing (Torres *et al.*, 2014; Cervini *et al.*, 2022).

Italy is considered at low risk for AFs contamination in peanuts, and this is an added value for the crop in this country. However, the spread of *A. flavus* in Mediterranean countries, including Italy, due to climate changes, may increase the risk of AF contamination in Italian peanuts, as has occurred in maize from 2003 (Piva *et al.*, 2006; Giorni *et al.*, 2007; Kos *et al.*, 2013; Battilani *et al.*, 2016; Moretti *et al.*, 2019). Applying suitable management practices along the peanut value chain is likely to reduce the risk of AF contamination (Chulze *et al.*, 2024).

Mechanistic models that consider interactions between *A. flavus*, host plants, and the environment to predict the risk of AF contamination are important tools in Integrated Pest Management (IPM) for sustainable agriculture. In particular, exploiting these models for sustainable agriculture promotes enhancement of agricultural practices, refinement of harvest strategies, and implementation of post-harvest measures, that aim to reduce potential risks for consumer exposure to AFs.

Some studies have aimed to predict AF contamination using crop growth simulation models. In Mali, Boken *et al.* (2008), through the CSM-CROPGRO-Peanut model, which is based on crop genetics, agricultural practices, soil data, and meteorological data, estimated peanut reproductive stages and crop yields. With this information Boken *et al.* (2008) performed regression analysis to correlate AF contamination measured post-harvest with weather conditions during the reproductive stages. Craufurd *et al.* (2006) carried out a similar study in Niger, using the CROPGRO-Peanut model to simulate crop growth and yield. Aflatoxin contamination at harvest was correlated with the fraction of extractable soil water (FESW) in the crop rhizosphere during the reproductive phase. Chauhan *et al.* (2010) in Australia also developed a predictive model based on the crop simulation model APSIM (Agricultural Production Systems

Simulator). This model uses the APSIM's peanut module to simulate crop growth. It calculates an aflatoxin temperature factor (ATF) during the last 40% of growth when soil water availability is below 0.20 (range 0-1), and the accumulated ATF generates an AF risk index (ARI). All of these models are empirical, and require recalibration for use outside their original geographic contexts, for example for use in the Mediterranean basin.

The present study aimed to develop a mechanistic model, as a flexible tool usable across different geographic regions without requirement for adjustments (Battilani and Camardo Leggieri, 2015). The mechanistic model AFLA-maize was adapted for the *A. flavus*-peanut pathosystem, following the successful application to AFLA-pistachio nuts (Battilani *et al.*, 2013; Kaminariis *et al.*, 2020). The first step was to study peanut growth stages, and then build a phenological model based on growing degree days (GDD). The model was then integrated with the model for the *A. flavus* infection cycle model, and was exploited to produce the prototype AFLA-peanut predictive model.

MATERIALS AND METHODS

Location of field studied

Between 2021 and 2023, meteorological data were collected from a total of 14 locations across Northern Italy provinces (Figure 1), including Ferrara (one location in 2021, six in 2022, two in 2023), Modena (one location in 2022), Cuneo (one in 2022), Verona (two in 2023), and Pordenone (one in 2023). The meteorological data from the 2022 and 2023 in the respective loca-

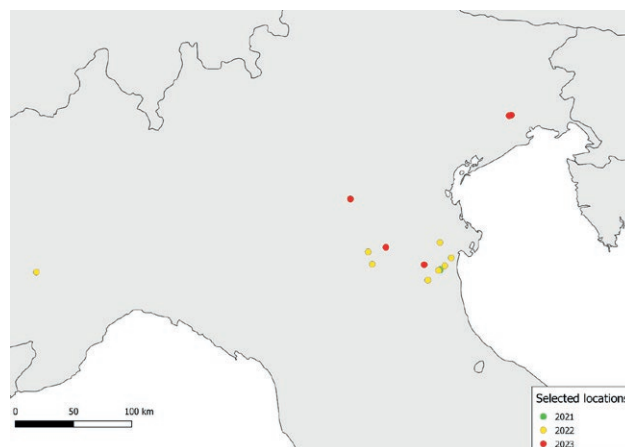


Figure 1. Geographical distribution of selected fields in Northern Italy across years (2021, 2022 and 2023).

tions were used as input to run the AFLA-peanut model. Among these production areas, four fields in the province of Ferrara (2021 and 2022), and one field in Pordenone (2023), were specifically selected to observe peanut growth stages for the peanut variety Lotos.

Lotos is commonly cultivated in Italy, and is characterised by early maturity and a crop cycle length of 120 to 130 d. Peanut season length is classified as “early” to 130 days, “medium” 133 to 139 d, “medium-late” 140 to 145 d, and “late-maturing” 146 to 155 d (Carter *et al.*, 2016; GRDC, 2018).

Meteorological data

Hourly data of air temperature (T, °C), relative humidity (RH, %), and rainfall (R, mm) were collected from the agro-meteorological network in Emilia Romagna region, from 1 January to 31 October during 2021, 2022 and 2023 (Table 1s, supplementary material). The Emilia Romagna region is virtually shared by a grid of squares, each of 5 km², with meteorological data delivered for each square (Arpae, 2024). These data come from all available sources, including meteorological stations and radar (Bottarelli and Zinoni, 2002). The proper squares were selected based on the locations of the monitored peanut fields. For the sampling points in other regions, meteorological data were provided by “Agrometeo Service” Image Line® and AgroNotizie®.

The meteorological data were used: i) to develop the peanut phenological sub-model included in the AFLA-peanut model, and ii) as input to run AFLA-peanut.

Growth stages

Crop phenology descriptions were based on field observations carried out every 2 weeks, from crop emergence to harvesting, during the complete peanut growth period (May to October). The growth stages were defined according to the BBCH scale (Meier, 2001), and after analysis of existing literature, the crucial peanut growth stages most susceptible for *A. flavus* infection were then indicated (Cole *et al.*, 1986; Pitt *et al.*, 1991; Horn, 2005).

The GDDs were calculated, starting from sowing date, for each observed peanut growth stage (Mcmaster and Wilhelm, 1997), using the following equation:

$$GDD_i = [(T_{\max,i} - T_{\min,i})/2] - T_{\text{base}}$$

where T_{\max} is the hourly maximum temperature, T_{\min} is the hourly minimum temperature, and T_{base} is the base temperature of 10°C.

T_{base} was set as the low threshold for peanut growth (Ketring and Wheless, 1989; Canavar and Kaynak, 2010; Kingra and Kaur, 2012). Collected data were assessed with literature sources (Banterng *et al.*, 2003; Canavar and Kaynak, 2010), leading to development of a crop phenology model for peanuts based on GDD (Canavar and Kaynak, 2010).

Predictive model

Commencing from the existing relational diagrams of AFLA-maize and AFLA-pistachio (Battilani *et al.*, 2013; Kaminiaris *et al.*, 2020), a new diagram was developed following the principle of “system analysis” (Lefelaar, 1993). The diagram was composed of different state variables linked in a coherent mathematical framework, which operates in a predictive model to generate a cumulative index for aflatoxin B1 (AFB1) contamination (AFB1-I). The predictive model, named “AFLA-peanut”, is a weather-driven model that predicts crop phenology and *A. flavus* behaviour based, on meteorological data (T, RH, and R).

RESULTS

Meteorological data

Data from selected locations are shown in Table 1s (supplementary materials). Temperatures in 2023 were characterised by high variability between locations. Nevertheless, during the first part of the year (January to April), and in September, temperatures were high (location daily thermal sum mean = 1012.9 °C, and 648.8 °C, respectively), compared to the same periods of 2021 (904.9 and 615.5 °C) and 2022 (888.5 and 595.8 °C). Nevertheless, Cavallermaggiore, in 2022, was the warmest location, compared to the other locations and years, from January to April (daily thermal sum = 949.7 °C). The opposite was recorded from May to July 2022, as daily thermal sum means were higher than in 2023 and 2021. For precipitation, high variability was observed between locations over the 3 years. However, drier periods occurred in 2022 and 2021 than in 2023, from May to July and during October. The exception was in Ostellato, in July of 2021 and in June of 2022. Similarly, in September of 2023, Bondeno and Cordovado had similar rainfall and were comparable to some of the other locations of 2022. In particular, during this month, the greatest amounts of precipitation were recorded in 2021. In 2023, Bondeno and Cordovado were the rainiest locations.

Peanut growth stages

GDD were computed for the main growth stages of peanuts, based on field observations conducted in 2021, 2022, and 2023 (Figure 2). The data collected were comparable to data reported previously for the early variety Florispan, with the same season length as Lotos, considered in the present study (Table 2s). For available data for late maturing varieties, required GDD increase from the beginning of flowering until harvest is approx. +20%.

Results of the relationships between GDD and BBCH are shown in Figure 2. Crop phenology can be split between vegetative and reproductive growth stages. The vegetative stages, from sowing (BBCH 0) to the beginning of flowering (BBCH 61), lasted for about 5 weeks, requiring approx. 425 GDD. The reproductive stage, which extends from flowering (BBCH 65) to harvest (BBCH 99), requires an additional 1239 GDD to be completed. Flowering was identified as the critical stage for *A. flavus* infection.

Predictive model

The infection cycle of *A. flavus* on peanuts is illustrated in the relational diagram in Figure 3. Inoculum source, which is not quantified in the model, represents the starting point of the cycle, considering that suitable environmental conditions influence the sporulation rate of the *A. flavus* (SpoR), promoting the production of spores. Subsequently, the spores produced on inoculum sources (SoI) are then dispersed according to a dispersal rate (DispR) and reach the peanut plants (DSoP). When the peanut crop is at a critical growth stage (GS) for *A. flavus* infection, from flowering, and the environmental conditions are suitable, the spores germinate, and the fungus grows on pegs and pods (GoPP), leading to infection of peanut seeds (IPS). These stages are regulated by the spore germination and growth rates, which are influenced by T, RH, and R. Once the seeds are infected,

the fungus may produce AFB1 (AFB1-I) according to an AFB1 production rate (AFB1R).

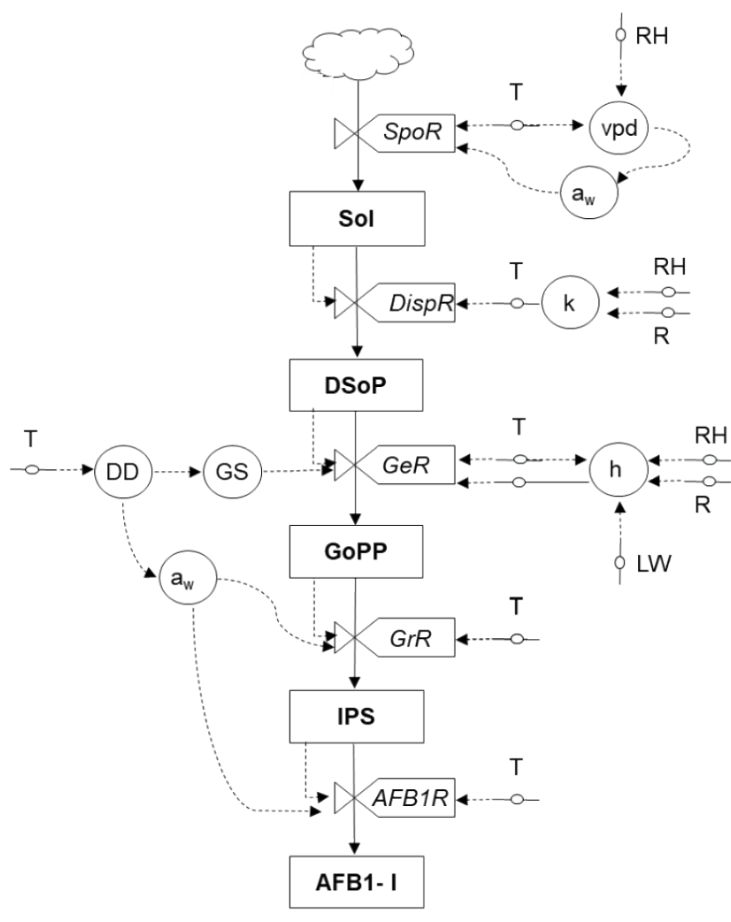
AFB1-I was computed daily, using hourly data collected in all the available meteorological data sources. Cumulative AFB1-I, from peanut flowering to harvesting, is the final output of the AFLA-peanut model. The AFB1-I index showed high variability across the 3 years and the locations considered in the present study (Table 1).

DISCUSSION AND CONCLUSIONS

Peanut production in Italy has increased in recent years, although AFB1 contamination has been only very rarely detected, and then only as traces (Crosta *et al.*, in preparation). However, increasing climate variability, with extreme events such as heat waves and droughts, attributed to climate change in the Mediterranean basin, underscore the need for a robust, weather driven-mechanistic model to predict AFB1 contamination risks (Battilani and Camardo Leggieri, 2015; Battilani *et al.*, 2016). Meteorological data collected in the present research has revealed distinct patterns across the years and locations studied, confirming the impacts of climate on weather dynamics at regional scale (Leggieri *et al.*, 2020). While drought conditions were more marked in 2021 and 2022, 2023 had more favourable precipitation for peanut growth. The locations selected for this had considerable rainfall differences and distribution throughout the growing seasons. AFB1-I, provided as output by AFLA-peanut, was characterized by high variability across years and locations, indicating the influence of meteorological data used as input, with temperature playing an important role, as has been previously observed for AFLA-maize and AFLA-pistachio (Battilani *et al.*, 2013; Kaminiaris *et al.*, 2020). In the present study, the model was transferred from the maize pathosystem to that for peanut, without modifying the fungal component, as was previously done for pistachio. Sensitivity analysis was therefore not applied because the results were con-

BBCH	0	9	24	61	62	65	66	73	79	85	89	99
Growth stage	Sowing	Emergence	4 th lateral shoot visible	Beginning of flowering	Peg formation	Flowering	Pegs penetrate the soil	Beginning of pods filling	Complete pods development	50% of pods are ripe	Complete maturity	Harvesting
GDD	0	92*	297	425	560	686	718	907	1304	1500	1759	1925

Figure 2. Crop phenology (described by the BBCH scale), mean GDD calculated for each growth stage, of the early peanut variety Lotos, observed in the fields of Ferrara and Pordenone from 2021 to 2023. $T_{base} = 10^{\circ}\text{C}$ was considered to calculate GDD.



Legend

Variables

Sol = Spores on Inoculum sources
 DSoP = Dispersed Spores on Peanut plant
 GoPP = Growth on peanut Pegs and Pods
 IPS = Infected Peanut Seeds
 AFB1-I = AFB1 in peanut seeds - Index

Intermediate variables

GS = Peanut Growth Stage
 DD = Degree Days
 vpd = Vapor pressure deficit
 k = Rain/humidity factor
 h = Rain/humidity/leaf wetness factor
 a_w = water activity

Rates

SpoR = Sporulation Rate
 DispR = Dispersal Rate
 GeR = Germination Rate
 GrR = Growth Rate
 AFB1R = AFB1 production Rate

Parameters

T = air Temperature
 RH = Relative Humidity
 R = Rain
 LW = Leaf Wetness

Figure 3. Relational diagram for the *Aspergillus flavus* infection cycle and aflatoxin production on peanuts.

sistent with those reported by Battilani *et al.* (2013) and Battilani *et al.* (2016).

Despite the differences in meteorological conditions between the three growing areas, GDDs from different sources and related to peanut growth stages agreed. This indicated that the peanut phenology model developed in the present study is reasonably robust (Banterng *et al.*, 2003; Canavar and Kaynak, 2010). For early season peanut varieties, 686 GDD were related to flowering (the critical stage for *A. flavus* infection), and 1925 GDD were related to peanut harvest.

The model presented here for AF contamination risk prediction is an improvement compared with existing empirical models, such as those by Craufurd *et al.* (2006), Boken *et al.* (2008), and Chauhan *et al.* (2010). The mechanistic approach of AFLA-peanut can adapt to diverse climatic profiles without the need for significant adjustments, positioning AFLA-peanut as a valuable tool

for both regional and international contexts (Battilani and Camardo Leggieri, 2015). The model’s flexibility and adaptability to environmental conditions are especially relevant for the Mediterranean environment, where climate affects traditional agricultural practices, and can threaten crop quality and safety (Battilani and Camardo Leggieri, 2015; Chulze *et al.*, 2024).

Results from the present study emphasize the need for thorough validation of the AFLA-peanut model. Georeferenced data on weather and AFB1 contamination from diverse peanut production regions, and additional data on peanut cropping systems, are important for achieving this aim. Such validation will provide important insights into the model’s prediction accuracy, and will strengthen its applicability in a broad range of environments.

The AFLA-peanut model’s ability to incorporate weather variability offers a strategic advantage for proactively managing AFs risks, which are increasingly chal-

Table 1. Summary data related to selected field locations in northern Italy in 2022 and 2023, with geographical coordinates, dates of sowing and complete maturity, and AFB1-I, the AFs cumulative indices generated as output by the prototype AFLA-peanut model.

Year	Location	Province ^a	Long	Lat	Sowing	Complete maturity	AFB1-I
2021	Ostellato	FE	12.063674	44.699471	10/05	30/09	7454
2022	Ostellato	FE	12.046193	44.695327	10/05	13/09	6018
	Lagosanto	FE	12.188809	44.788623	10/05	07/09	4552
	Volania	FE	12.116027	44.728864	10/05	10/09	6209
	Dosso	FE	11.328936	44.759730	10/05	04/10	852
	Argenta	FE	11.926363	44.623563	11/05	22/09	2290
	Mezzogoro	FE	12.071466	44.911498	09/05	16/09	3707
	Finale Emilia	MO	11.287771	44.856394	19/05	29/09	828
Cavellermaggiore	CN	7.681548	44.715500	07/05	28/09	532	
2023	Bondeno	FE	11.481934	44.887692	30/05	29/09	5465
	Ostellato	FE	11.891678	44.743345	27/05	21/09	8188
	Bovolone	VR	11.109327	45.270235	14/06	03/10	1708
	Bovolone	VR	11.111219	45.268570	14/06	03/10	1708
	Cordovado	PN	12.914613	45.870081	27/05	03/10	3408

^a FE = Ferrara, MO = Modena, CN = Cuneo, VR = Verona, and PN = Pordenone

lenging under the Mediterranean region's shifting climate conditions. As climate change accelerates, extreme weather events are expected to intensify, directly affecting crop health and AF contamination (Battilani *et al.*, 2016; Chulze *et al.*, 2024). These climate changes pose threats to food safety, and to the economic stability of agricultural sectors and to food security. *Aspergillus flavus* outbreaks reduce crop yields and restrict market access for affected produce (Moretti *et al.*, 2019) in areas where peanuts contribute significantly to farmer income.

AFLA-peanut could be an important tool for safeguarding peanut supply chains and consumer health, by providing early risk alerts, once its robustness is confirmed through validation. This has already been demonstrated for AFLA-maize and AFLA-pistachio (Battilani *et al.*, 2013; Kaminiaris *et al.*, 2020). By anticipating contamination risks, these models help mitigate adverse health effects and economic losses associated with the disposal of unsafe product batches. Implementation of costly interventions could be justified in cases of high AF contamination risk.

Future research should focus on validating the AFLA-peanut model using diverse datasets with a broad range of AFB1 contamination to further assess the model's predictive performance. In subsequent developments, the model output could be a foundation for a comprehensive Decision Support System (DSS). This would integrate additional variables, such as peanut genetic, agricultural, and soil factors, to enhance AF risk prediction and support improved peanut safety management within the framework of sustainable agriculture.

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