SCIENTIFIC REPORT submitted to EFSA

Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change[1]

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ABSTRACT

Aflatoxin (AF) contamination in maize is of worldwide importance. *Aspergillus flavus* and *A. parasiticus* are the principal fungi responsible for AF production. Based on the current literature, AFs are not considered a problem in wheat and rice at harvest and no data were found on aspergilli-wheat/rice interactions in the field. Data on the effects influencing the development of *A. flavus* and *A. parasiticus* on maize and maize kernel at harvest were collected; however data on *A. parasiticus* and AFB_2-G_1-G_2 were not sufficient for further use in predictive modelling. Thus, a model was developed to predict the risk of AFB_1 contamination, due to *A. flavus*, in maize at harvest and further adapted to wheat and rice as host crops. The Joint Research Centre of the EC provided a database with mean daily temperatures during emergence, flowering and harvesting of maize, wheat and rice. Meteorological data (temperature, relative humidity and rain) obtained from the LARS weather generator, were used as input for the modelling of crop phenology and *A. flavus* behaviour. The output was designed at a 50 x 50 km scale over the European territory and generated over 100 years, in three different climate scenarios (present and A2 and B2 storylines, or + 2 °C and + 5 °C scenarios, proposed by the Intergovernmental Panel on Climate Change). Predictions showed a reduction in season length and an advance in flowering and harvest dates leading to an enlargement of the crop growing areas towards north EU, mainly for maize and rice, because earlier ripening could occur in these areas. The risk of *A. flavus* contamination was expected to increase in maize, both in the + 2 °C and + 5 °C scenarios, to be very low in wheat and to be absent in rice. Results were discussed and recommendations were made on data collection and prevention measures on AF risks.

SUMMARY

The impact of climate change has been identified as an emerging issue for food and feed safety. With its mandate to identify emerging risks in food and feed production, EFSA's Emerging Risks Unit identified changing patterns in mycotoxin contamination in cereals such as wheat, maize and rice, due to climate change as a potential emerging hazard. In particular, aflatoxins (AFs) which are frequent in tropical and sub-tropical areas may become a concern in the EU. *Aspergillus flavus* and *A. parasiticus*, the main AF producers, are xerophilic fungi. With climate change and expected increasing temperature and decreasing rain, these fungi may find conditions that are more suitable for their development.

An inventory and modelling of the factors influencing the emergence of AFs in maize, wheat and rice crops in EU due to climate change, as well as the production of maps to highlight predicted AF contamination in these crops was requested. Therefore, the aim of the current study was to evaluate the scientific literature related to AF contamination in wheat, maize and rice, and to develop predictive models and draw maps of potential AF contamination in these crops in EU.

A literature review was performed to provide an accurate "state of the art" summary regarding the role of ecological factors on the growth and metabolic activity of *A. flavus* and *A. parasiticus*. Other factors dealing with aspergillus-crop interactions, crop phenology, climate change effects/scenarios and predictive models were also investigated. This review followed the principles of the systematic literature review as described by EFSA (2011). The main sources of information were CAB abstracts on OVIDSP; ISI Web of Sciences and Scopus. In these databases it was possible to review papers from 1978 onwards. All relevant papers were assessed in full (title, abstract and text) to extract all the required information. These papers were stored in an EndNote database and all the data describing modelling, meteorology and climate change scenarios, crop phenology predictions and AF indexes were stored in MS Excel tables.

Based on these results, together with data on *A. flavus* produced by the consortium during the course of this project, a predictive model was developed for *A. flavus* growth and AFB_1 production on maize.

Aspergillus parasiticus and aflatoxins other than AFB_1 were not included in the modelling because data were limited and not sufficient for further use in predictive modelling. The *A. flavus*-AFB_1 model was then linked to crop phenology data in maize, wheat and rice. The crop phenology database developed by the Joint Research Centre of the European Commission (JRC) at the EU scale was used and further adapted by the MODMAP consortium (i.e. the authors of the MODMAP project) to the specific needs of the project.

The *A. flavus*-AFB_1 model, linked with crop phenology, was based on daily meteorological data to provide AF indexes, also designated as meteorological risk indexes for AFB_1 contamination in maize, wheat and rice during harvest at the European level. Daily meteorological data were obtained by the consortium using the LARS weather generator. A series of 100 years, intended as 100 runs of the LARS model, was produced for the three selected climate change scenarios (i.e. actual, + 2 °C and + 5 °C). For the mapping of these data, crucial years were selected based on the output data, considering temperature and rainfall variability between years.

The risk of AF contamination was predicted in each of the three climate change scenarios using *A. flavus*-AFB_1 model, predicted crop flowering and harvest dates, and meterological data. Results on climate, crop phenology and AF risks were used for statistical analysis and mapping.

The literature search pointed out a lack of data on the ecology of *A. parasiticus* and its role in AF contamination in maize, and a lack of data as well on AF contamination in wheat at harvest. No literature was found on AF contamination in rice at harvest and on wheat and rice interaction with *A. flavus*.

Surveys on AF contamination in maize were found to be focused on AFB_1 whereas few data were available regarding AFB_2 and AFGs. Based on the data collected in Italy, AFB_1 represented 90 % of the total of AFs detected in maize in the last ten years, suggesting that predictions of AFB_1 risk provided a reasonably good picture of the risk of total AFs.

Predictions showed a reduction in season length and an advance in flowering and harvest dates for all the crops considered; this could allow an enlargement of the crop growing areas towards the north of EU, mainly for maize and rice, because earlier ripening would then be possible in these areas.

According to the results of this project, the risk for AFs contamination is expected to increase in maize, mainly in the + 2 °C scenario. In this scenario, a clear increase in AF risk was shown in typical European agricultural areas such as in the centre and south of Spain, the south of Italy and in the Balkans, including Turkey (European Turkey only). Finally, a 5 and 10 days advance was predicted in flowering and harvesting dates respectively, implying no change in the agricultural practices management.

The + 5 °C scenario depicted a completely different situation with a considerable enlargement of AF risk areas and an overall decrease in AF risks, therefore, the extended areas of south-east Europe would present medium to low risks. In this scenario, a consistent advance in flowering and harvesting dates was estimated at 10 and 15-20 days, respectively, implying possible changes in the agricultural practices management.

In summary, in the + 2 °C scenario, higher levels of contamination are expected in the areas where maize is currently grown whereas in the + 5 °C scenario, levels of contamination are predicted to be lower but risks are expected to be wider and enlarge towards northern EU countries.

Considering the different climate change scenarios and matching them with the calculated AF risks and actual data on crop production, the following scenarios were depicted: (i) high AF risk in the

southern EU countries that are not in the current main maize crop production area (i.e. in central and south of Spain, central and south of Italy, Greece, north and south-east of Portugal, Bulgaria, Albania and Cyprus); (ii) low and medium AF risks in the four main maize productive countries (i.e. in Romania, France, Hungary and north east of Italy, all accounting for 73 % of the present total of the EU-27 production); (iii) low AF risks in northern European countries, that currently are in the safeguard zone regarding AF risk due to their climatic conditions; (iv) very low AF risks in Europe for wheat and rice.

The risk maps produced by the *A. flavus*/AF model were proposed to be used as a communication tool to reinforce prevention of AF risks by identifying priority locations for intervention. The predictions confirmed that maize is the cereal crop of concern and that both human and animal populations could be exposed to a high AF risk, at least in some EU regions. Wheat would present a negligible AF risk and rice no risk at all. However, the estimated AF risk cannot be quantitatively correlated to EU legal maximum levels for AF contamination. Nevertheless, it is suggested to gather data to understand the impact of the application of good agricultural practices in the field and good post-harvest management practices to control and prevent potential AF risks.

KEY WORDS

Aspergillus flavus, *Aspergillus parasiticus*, maize, wheat, rice, aflatoxin B_1, risk maps

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BACKGROUND

The impact of climate change has been identified as an emerging issue for food and feed safety (Miraglia et al., 2009). With its mandate to identify emerging risks in food and feed sectors, EFSA's Emerging Risks Unit has identified changing patterns in mycotoxin production in cereals, wheat, maize and rice, due to climate change as a potential area of concern. Therefore, the Emerging Risks Unit is seeking scientific information, based on models and scenarios, to predict the potential growth of mycotoxins in the EU due to climate change. This work should support risk assessment activities of EFSA's Panels.

Food mycotoxins are metabolites of plant pathogenic fungi and affect 25 % of the world's food crops (Charmley et al., 1995). Aflatoxin B_1 is the most frequently reported mycotoxin in the RASFF database (Kleter et al., 2009). The level of aflatoxin B_1 contamination is high in maize, wheat and rice (RASFF, 2009), all of which are highly cultivated in and imported into the EU (Eurostat, 2009). This call will focus on the prediction of the potential spread of both total aflatoxin (B_1, B_2, G_1 and G_2) and specifically aflatoxin B_1 producing fungi in maize, wheat and rice due to climate change.

It is difficult to determine the potential risks of emerging mycotoxin producing microorganisms because their development is influenced by several interacting factors such as microclimatic conditions (e.g. local temperature and humidity), use of fertilisers, and insect attacks – all of which are modulated by climate change. In addition, the response of mycotoxin producing microorganisms to environmental conditions is species-specific (Murphy et al., 2006).

Based on the most relevant climate change scenarios for agriculture and food safety and on a complete inventory of the factors influencing the growth/spread of the targeted fungi, models are required to predict the spread of aflatoxin producing fungi, such as *Aspergillus flavus* and *A. parasiticus,* in maize, wheat and rice plants in the EU. *Aspergillus flavus* is ubiquitous, favouring the aerial parts of plants (leaves, flowers) and produces B aflatoxins (B_1 and B_2). *Aspergillus parasiticus* produces both B and G aflatoxins (B_1, B_2, G_1 and G_2), is more adapted to a soil environment and has more limited distribution.

The project shall focus on the growth of aflatoxin producing fungi at the pre-harverst stage only and highlight potential gaps in knowledge. Potential contamination events during transportation, storage and trading are thus outside the scope of this call.

TERMS OF REFERENCE

EFSA is seeking proposals to make an inventory of the factors influencing the emergence of aflatoxins in maize, wheat and rice plants in the EU due to climate change. These data will then need to be used to build predictive models and draw maps of potential growth of emergence of aflatoxin producing microorganisms in the EU.

In particular, the contractor is expected to develop the following work packages (WP):

WP1: Inventory and listing of the factors influencing the growth/spread of aflatoxin producing fungi in maize, wheat and rice plants in the EU at the pre-harvest stage. Collate data in Endnote and Access databases. This inventory will be based on the available scientific knowledge (scientific literature, reports and expert network from EU and national scientific projects, etc.). The keywords, sources of information and databases used will be described and justified; Criteria to select relevant factors (i.e. factors influenced by climate change) will be defined and justified. For these factors, available data and gaps will be identified and highlighted.

WP2: Selection of climate change scenarios; data screening and cleaning. A selection of a minimum of two climate change scenarios will be proposed and justified as well as the methodology used to screen

data and clean the database (exploration of statistics, identification of double data, harmonisation of records, outlines of the exclusions, handling of missing values and identification of colinearities).

WP3: Data modelling and mapping. The models selected to predict the growth of aflatoxin producing fungi (both total aflatoxins (B_1, B_2, G_1, G_2) and specifically aflatoxin B_1) in maize, wheat, and rice plants as well as the maps to be drawn will be thoroughly described (selection criteria, model definition, outputs and simulation). Two types of models will be proposed to predict the growth of *A. flavus*, an airborne fungus, and *A. parasiticus*, a soil-borne fungus.

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1. INTRODUCTION

The impact of climate change has been identified as an emerging issue for food and feed safety (Miraglia et al., 2009). With its mandate to identity emerging risks in food and feed sectors, EFSA's Emerging Risks Unit has identified changing patterns in mycotoxin production in cereals, wheat, maize and rice, due to climate change as a potential area of concern.

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Food mycotoxins are metabolites of plant pathogenic fungi and affect 25 % of the world's food crops (Charmley et al., 1995). Aflatoxin B_1 is the most frequently reported mycotoxin in the RASFF database (Kleter et al., 2009). The level of aflatoxin B_1 contamination is high in maize, wheat and rice (RASFF, 2009), all of which are highly cultivated in and imported into the EU (Eurostat, 2009). This report focus on the prediction of the potential spread of both total aflatoxin (B_1, B_2, G_1, G_2) and specifically aflatoxin B_1 producing fungi in maize, wheat and rice due to climate change.

Based on the most relevant climate change scenarios for agriculture and food safety and on a complete inventory of the factors influencing the growth/spread of the targeted fungi, models are required to predict the spread of aflatoxin producing fungi, such as *Aspergillus flavus* and *A. parasiticus,* in maize, wheat and rice plants in the EU.

The report focus on the growth of aflatoxin producing fungi at the pre-harvest stage only and highlight potential gaps in knowledge.

1.1. The Aspergillus section flavi

Members of *Aspergillus* section *flavi* are widely distributed in nature. They are regularly isolated from soils, particularly those from tropical and subtropical areas, from forage and decaying vegetation, from stored seeds and grains and from various types of food products. They contribute to decomposition processes and some of them are pathogenic to insects and, for example, *A. flavus* and *A. parasiticus*, to higher animals including man (Raper and Fennell, 1965).

Aspergillus flavus and *A. parasiticus* are closely related fungi which can contaminate primary agricultural products in the field, during harvest, in storage, and during processing (Diener et al., 1987). Strains with shorter stalks, borne from the substrate and bearing persistently yellow-green heads were placed in the *A. flavus* series and segregated as two species: *A. flavus* Link and *A. parasiticus* Speare. The two species were differentiated, in part, by their colour and relative conidiophore[2] lengths, but primarly by the character of their sterigmata[3]: *A. flavus* was typically biseriate and *A. parasiticus* uniseriate (Raper and Fennell, 1965).

There is also another characteristic that helps distinguish between these two species: *A. parasiticus* appears to be adapted to a soil environment, being prominent in peanuts, whereas *A. flavus* seems adapted to the aerial and foliar environment, being dominant in maize, cottonseed, and tree nuts (Diener et al., 1987). More recently different results were found in field surveys (Abbas et al., 2005; Costa et al., 2009; Pildain et al., 2005; Pushvinder and Desai, 2006).

Researchers have frequently failed to distinguish between the two species in their studies, (Kurtzman et al., 1987) addressed this problem through comparisons of deoxyribonucleic acid (DNA) relatedness and found sufficiently high complementarities among the two taxa to conclude that they were co

[2] Asexual reproduction in is by the formation of conidia, which are borne on specialized stalks called conidiophores. The morphology of these specialized conidiophores is often distinctive of a specific species and can therefore be used in identification of the species.

[3] Sterigmata are slim projecting parts of the conidiophore that carries the spores.

specific. In fact it has been demonstrated that the *A. flavus* cluster is 96 % identical to that of *A. parasiticus* (Cary and Ehrlich, 2006).

The production of mycotoxins, a key character of these fungi, could be useful to separate strains of the *A. flavus* group. It is now generally accepted that *A. flavus* usually only produces aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2), but is also capable of synthesising cyclopiazonic acid (CPA), a mycotoxin confirmed as being present in the batch of contaminated groundnuts which killed turkeys in 1960 (Turkey 'X' disease) (Smith, 1997). On the other hand, *A. parasiticus* often produces all four of the primary aflatoxins (AFs): this group of mycotoxins comprises AFB_1, B_2, G_1 and G_2 (D'Mello and Macdonald, 1997; Diener et al., 1987). However, recent studies demonstrated that certain strains of *A. flavus* can also be able to produce AFG_1 and AFG_2. For example in a study with *A. flavus* isolates from Africa and America, it was found that from 40 to 100 % of African strains were able to produce also AFG_1 depending on the media used while none of the American strains were (Cotty and Cardwell, 1999). However, in both species of *Aspergillus* section *Flavi*, there are strains that are non-aflatoxigenic (Smith and Moss, 1985).

Most of the *Aspergillus* species grow above the 25° latitude north and south, with a high occurrence between 26 ° and 35° latitudes, while it is uncommon in latitudes above 45° (Klich, 2007). Temperatures suitable for growth of *A. flavus* are varying with a minimum from 10 to 12.8 °C, a maximum between 43 °C and 48.8 °C and an optimum near 33.8 °C were stated. Proper water activity (a_w) for growth are for instance 0.82 at 25.8 °C, 0.81 at 30.8 °C and 0.80 at 37.8 °C (Pitt and Hocking, 2009).

1.1.1. Aspergillus flavus infection cycle

Aspergillus section *Flavi* can easily survive and colonize soil and organic debris associated with plant residues. Although soil serves as the primary habitat of *A.* section *Flavi*, little is known about the life cycle of these fungi in soil. *A. flavus* is capable of surviving and overwintering in plant residues as mycelium[4] or sclerotia[5] that in turn serve as the source of new conidia[6] to start the infection cycle on new host plants. The major factors that influence soil population of these fungi are soil temperature and moisture.

The *A. flavus* life cycle can be divided into two major phases: the colonization of plant residues in soil and the infection of crop tissue. At the beginning of the growing season, when suitable environmental conditions arise, sclerotia and conidia, the overwintering structures, germinate into mycelia that produce numerous conidiophores and release conidia into the air. This new inoculum is vectored by insects or carried by wind to begin colonization and infection of the freshly planted crops. Both wind dispersal and insect damage are associated with *Aspergillus* infection in various crops such as maize, cottonseed, peanuts and tree nuts (Horn, 2005; Northolt, 1979; Payne, 1998; Scheidegger and Payne, 2005). Factors affecting infection, apart environmental conditions, are the amount of spores in the field and plant susceptibility (depending on crop, variety and health status), cropping system and insect population, (Northolt, 1979). Timing of insect damage influences mycotoxin levels as fungal growth depends on moisture levels of the crop (Dowd et al., 2005). In maize, significant infection and

[4] Mycelium (plural mycelia) is the vegetative part of a fungus, consisting of a mass of branching, thread-like **hyphae**. Fungal colonies composed of mycelia are found in soil and on or within many other substrates. A typical single **spore** germinates into a homokaryotic mycelium, which cannot reproduce sexually; when two compatible homokaryotic mycelia join and form a dikaryotic mycelium, that mycelium may form fruiting bodies such as mushrooms. A mycelium may be minute, forming a colony that is too small to see, or it may be extensive. It is through the mycelium that a fungus absorbs nutrients from its environment.

[5] A sclerotium (plural *sclerotia*) is a compact mass of hardened fungal mycelium containing food reserves. One role of sclerotia is to survive environmental extremes. In some higher **fungi** such as ergot, sclerotia become detached and remain dormant until a favorable opportunity for growth.

[6] Conidia, sometimes termed conidiospores, are asexual, non-motile spores of a fungus. They are cells genetically identical to the parent, can develop into a new organism if conditions are favorable, and serve in biological dispersal.

AFs production does not occur until the kernel moisture is below 32 % (Payne, 1998). Aflatoxin can continue to be produced until the kernel moisture reaches 15 % (Anonymous, 2003). If insect damage takes place after the crop has dried to a level that does not support fungal growth, it is expected that mycotoxin levels will remain low unless subsequent rainfall re-water the crop (Dowd et al., 2005). Apart from timing of insect damage, timing of harvest also influences AFs levels in the crop, with delayed harvest resulting in increased AFs levels. Influences of delayed harvest are most severe when crops are caught by rain just prior to or during harvest (Cotty and Jaime-Garcia, 2007).

1.1.1.1. Aspergillus in maize cultivation

The infection cycle of *A. flavus* was thoroughly studied with maize and it is summarized in Figure 1.

It follows what has been described in the previous section; the susceptible stage of maize crops is flowering, silks browning in particular, when air-borne or post-borne spores optimise their efficiency.

Aspergillus flavus can infect grains post-harvest and it can result in an increase in AFs contamination if the drying and storage phases are poorly managed. Post-harvest infection is strictly related to fungal presence in field, in fact, kernels colonized by *A.* section *Flavi* represent the inoculum for infection during the storage period. So, fungal infections in the field may be an important source of mycotoxin formation later in the supply chain.

Aspergillus flavus can grow and produce mycotoxins down to 0.73 and 0.85 water activity respectively (Sanchis and Magan, 2004). This corresponds to 8-12 % and 17-19 % moisture content (MC) in maize (Battilani et al., published on line; Battilani et al., 2007). Usually maize is stored in silos at 14 % MC. Inefficient drying or water ingress can cause pockets of wetter grain resulting in a higher MC (Magan and Aldred, 2007).

In stored grain ecosystems, the most important abiotic conditions identified which influence growth and mycotoxin production are water activity, temperature and, when grain is moist, gas composition (Guynot et al., 2003; Magan et al., 2004). In particular, interactions between these factors can determine whether contamination increases and mycotoxins are produced. Overall, the efficacy of controlled atmospheres × a_w showed that treatment with 25 % CO_2 could be sufficient to efficiently reduce *A. flavus* development but at least 50 % CO_2 is required to obtain a significant reduction of AFs synthesis (Giorni et al., 2008). Apparently, the predicted variations in CO_2 due to climate change (a few per cent units) will not affect *A. flavus* growth and AFs production.

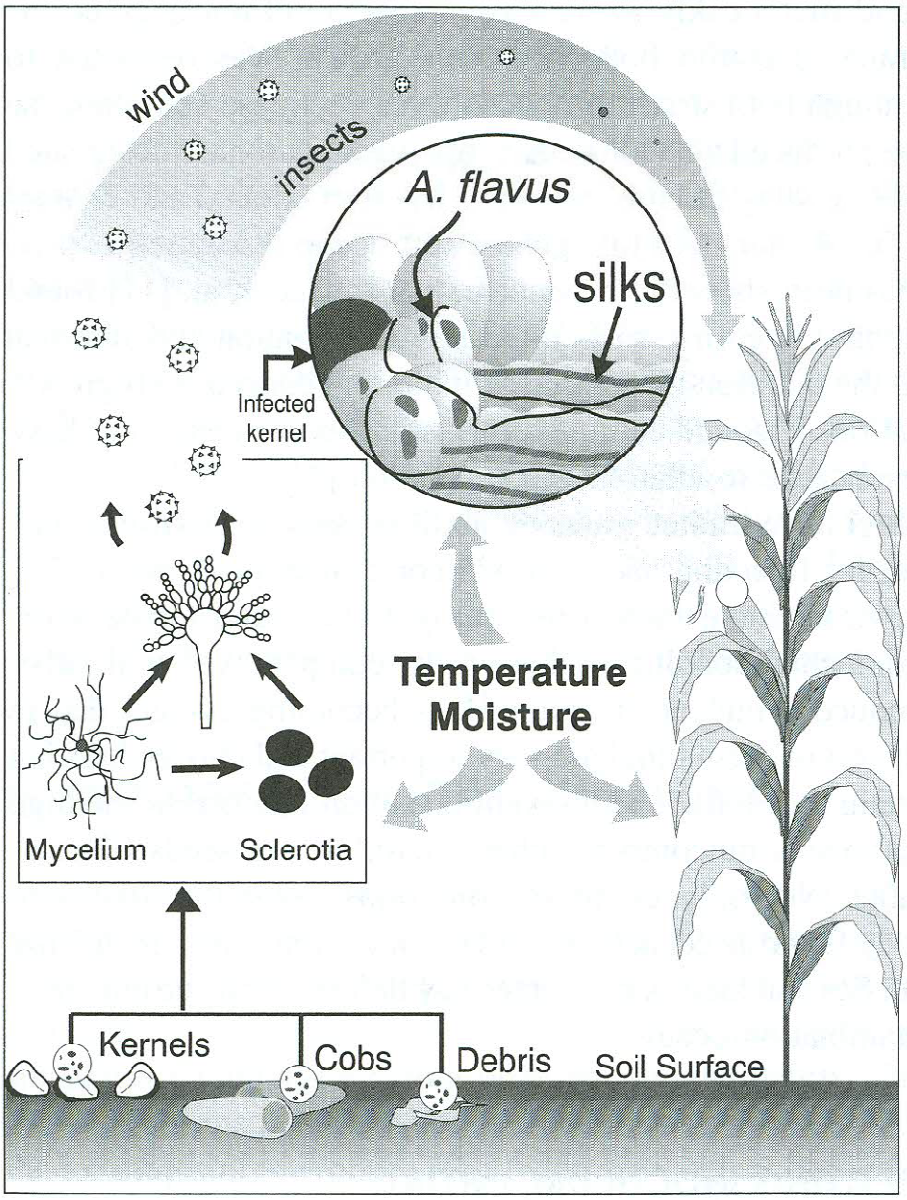

Figure 1: Schematic diagram showing the infection cycle of *A. flavus* in maize (Anonymous on line)

1.1.1.2. Aspergillus in wheat cultivation

Although several mycotoxins can be produced by wheat fungal diseases, the occurrence of AFs is mainly due to the infection of *A. flavus* and *A. parasiticus* of which the seasonal epidemiology in relation to wheat cropping patterns is hardly known; aspergilli have been mainly associated with infection of maize and cotton and the host-pathogen interaction was mainly studied in these crops. Because *A. flavus*-wheat interaction is hardly unknown, information analysis should focus on the factors that influence wheat development and growth, which are also relevant for *Aspergillus* infection and AFs production. The only option is to assume that processes and factors are similar to that in maize, in which both *Aspergillus* and AFs are seen more often. Several papers do describe the infection route of *A. flavus* on maize (Abbas et al., 2009; Payne, 1998; Scheidegger and Payne, 2005),

which is comparable to the infection route of *Fusarium* spp. (i.e. infection takes place primarily during flowering and through insect damage). It could be assumed that the wheat infection routes for these two fungal species may also be comparable. With maize, the flowering period is likely to be most critical for fungal infection and weather conditions up to harvest strongly affect AFs formation (Payne, 1992). Therefore, models that can predict the timing of wheat flowering and the period up to wheat harvest are probably most relevant in this regard.

Wheat infection by *Fusarium* takes place during flowering and depends on weather variables and agronomic factors. Several regression and mechanistic models have been developed for *Fusarium* spp. in wheat (Prandini et al., 2009). These models can be considered to check the approach followed and to adapt to *Aspergillus* infection using the weather and agronomic factors indicated as relevant. The values for the parameters used, however, need to be adjusted for *A. flavus* by incorporating their specific fungal parameters (like minimum and optimum growth temperature) as well as weather conditions and agronomic factors specific for *Aspergillus* growth and AFs production.

Also, these fungi have been associated with postharvest conditions in wheat, that are known to have major impact on *Aspergillus* infection and growth (Sharma et al., 2007). Postharvest contamination with *Aspergillus* originates from infection of the crop in field.

Although wheat grains are less susceptible to mycotoxin formation due to their smaller kernel size than larger grains such as maize, AFs can be produced when wheat is stored under high moisture and temperature conditions (Smith and Moss, 1985). Therefore, the initial infection with *Aspergillus* spp. in the field is important and potential pre-harvest infection should be studied.

1.1.1.3. Aspergillus in rice cultivation

Among the toxigenic fungi reported as common contaminants of rice produced in Europe, the species that produce AFs, namely *A. flavus* and *A. parasiticus*, have not been reported yet. On the other hand, species that produce other mycotoxins such as fumonisins, possible carcinogenic compounds to humans, classified in group 2B by IARC (1993), belonging to the genus *Fusarium,* most frequent *F. proliferatum* and *F. fujikuroi* (Leslie and Summerell, 2006), have been often isolated from rice seeds and plants and were related to a rice fungal disease called bakanae (Moretti et al., 2007). Therefore, the seasonal epidemiology of the *Aspergillus* species that produce AFs in relation to rice cropping patterns is unknown. However, from the knowledge available on maize, it can be assumed that perhaps also on rice the contamination can take place at flowering and increases in contamination during ripening. On the other hand, these fungi have been associated worldwide with postharvest conditions, more conducive for *Aspergillus* infection and growth (Pitt and Hocking, 2009). Nevertheless, fungal infections in the field has to be taken into account as an important source of mycotoxin formation later in the supply chain (Pitt and Hocking, 2009).

Rice is commonly dried after harvesting. Due to inappropriate storage conditions, rice can become an ideal substrate for AFs producing fungi. The contamination of the matured crops occurs by the exposure to warm and moist conditions either on field or in the storage facilities, where even initially dry seeds are susceptible to fungal contamination.

Rice is harvested at moisture content between 16 % and 28 % depending on the harvest technique and is dried to a content of 13.5 % (Brooker, 1992). When rice is exposed to high humidity and appropriate temperature, AFs can be produced. Therefore, it is important to evaluate the possible final contamination of AFs in rice and by-products. To this respect, the detection of AFs in rice has arose a growing interest with an increasing number of reports worldwide, showing a relevant level of contamination of rice samples by AFs (Kodani et al., 2002; Liu et al., 2006; Mazaheri, 2009; Park et al., 2005; Reddy et al., 2009; Sales and Yoshizawa, 2005; Zare et al., 2008). Moreover, in 2006 a case of aflatoxin M_1 contamination has been found in milk in Europe, caused by rice derived feed

contaminated with AFs up to 22 µg/kg. Feed components, namely by-products of basmati rice production, have been found to be contaminated up to 154 µg/kg (Nordkvist et al., 2008).

Information analysis should focus on the factors that influence rice development, growth, and storage which are also relevant for *Aspergillus* infection of rice and AFs production. However, a thorough analysis of fungal infection of rice and factors involved is hindered by a total lack of information about *Aspergillus* infection of rice under field conditions. The only option is to assume that processes and factors involved are similar to that cited in maize, with the flowering period as the most critical for fungal infection and weather conditions up to harvest strongly influencing AFs formation (Cotty and Jaime-Garcia, 2007). Therefore, models that can predict the timing of rice flowering and the period up to rice harvest are considered most relevant in this regard.

1.1.2. Aflatoxins

Aflatoxins acquired their names from the blue or green fluorescence that they exhibit when exposed to ultraviolet light (366 nm) on silica gel thin layer chromatograms (Hartley et al., 1963). The AFs belong to the B and G groups; in addition, AFM_1 and AFM_2 have been identified in the milk of dairy cows consuming AFB_1 and AFB_2 from contaminated groundnut meal (Van Egmond, 1989).

There are many gaps in the understanding of the coordinated global regulation of toxin formation, of the signal transduction pathways underlying primary and secondary metabolism, of the biotic and abiotic factors that affect toxin formation, and of the interactions of mycotoxigenic fungi and their host plants during infection (Bhatnagar et al., 2006). There are many theories about the meaning of AFs production by fungi, but nothing has been clearly demonstrated. Aflatoxins could be a defence response by fungi to stress, a way to protect fungi from UV (ultraviolet) damage, by-products of primary metabolism, necessary to increment fungal fitness or able to provide protection from predators for reproductive structures such as conidia and sclerotia (Cary and Ehrlich, 2006; Magan and Aldred, 2007).

Studies determined that AFs are synthesized by a polyketide metabolic pathway and that genes of both *A. flavus* and *A. parasiticus* linked to the AFs biosynthetic pathway are clustered (Bhatnagar et al., 2004; Chang et al., 2004).

1.1.3. Toxicity of aflatoxins

Aflatoxins may act as acute toxins (Platanow, 1964), carcinogens (Platanow, 1964; Wogan et al., 1971), teratogens (Ellis and di Paolo, 1967) and mutagens (Ong, 1975; Wong and Hsieh, 1976).

Animals demonstrate varying susceptibilities to AFs toxicity, which may be attributed to genetic (species, sex, breed and strain), physiological (age, nutrition, other diseases, presence of other toxins) and environmental (climate, husbandry, management) factors (Bradburn et al., 1993). Aflatoxins are primarily potent hepatotoxins, causing aflatoxicoses in humans and animals. Aflatoxicosis primarily attacks the liver causing necrosis, cirrhosis and carcinomas, and it does cause other health effects. Acute symptoms include vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and cerebral oedema (USDA, 2006). They occur in farm animals, both as a chronic disease characterised by an impairment of resistance and immune responsiveness, which results in a reduction in growth rate and feed efficiency and, as acute poisoning, characterised by severe clinical disease, liver tumours and death (Logrieco et al., 2003).

For humans, AFs is predominantly perceived as an agent promoting liver cancer, although lung cancer is also a risk among workers handling contaminated grain (Kelly et al., 1997). The risk of cancers due to the exposure to AFs is well established (Gorelick et al., 1993) and is based on the cumulative lifetime dose (Williams et al., 2004). However, the possible role of the immune system with respect to the incidence, severity and outcome of infectious diseases in developing countries leads to expect that

AFs may also affect the epidemiology of many diseases and health risks in those countries where the toxin is uncontrolled (Williams et al., 2004). In particular, it has been observed a strong synergy between AFs and hepatitis B and C virus (Groopman, 1993) and also with the degree of stunting and underweight in young children (Gong et al., 2002).

Because of their mutagenic, teratogenic, and carcinogenic potency, AFs are classified within Group 1, as compounds carcinogenic to humans (IARC, 1993).

1.1.4. European legislation

The European Commission (EC) established maximum levels for AFs presence in food (EC 2006[7] amended by EC 2010[8]) and feed EC 2003[9]. These limits have been established to provide an adequate margin of safety to protect human (Table 1) and animal health.

The maximum level was fixed at 5 µg of AFB_1 and 10 µg of AFB_1+AFB_2+AFG_1+AFG_2 per kg of maize and rice destined to human consumption and at 2 µg and 4 µg per kg of all cereals and their derived products. Regarding animals, the limit was fixed at 0.02 mg/kg of feed material with the exception of that destined to dairy animals; because of the possible carry over in milk, the limit is fixed to 0.005 mg/kg. A limit of 0.050 µg/kg of milk was fixed for AFM_1.

Due to these limits, analyses are currently carried out on the imported products in the EU and some studies have shown AFs contamination largely exceeding the legal limit (Fredlund et al., 2009; Reiter et al., 2010).

Table 1: Maximum levels of aflatoxin in cereals and milk (EC 2006[7])

Commodity	Maximum levels (µg/kg – ppb)		
	B_1	B_1+B_2+G_1+G_2	M_1
Maize and rice to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5.0	10.0	
All cereals and all products derived from cereals, including processed cereal products	2.0	4.0	
Processed cereal-based foods and baby foods for infants and young children	0.10		
Infant formulae and follow-on formulae, including infant milk and follow-on milk			0.025
Dietary foods for special medical purposes intended specifically for infants	0.10		0.025
Raw milk, heat-treated milk and milk for the manufacture of milk-based products			0.050

1.2. Occurrence of aflatoxins

In this section, surveys about AFs contamination in cereals produced in Europe have been considered. These data are useful to describe the actual situation and to point out lack of data. Moreover, all data available have been used later for model validation.

[7] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5-24.

[8] Commission regulation (EU) No 165/2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. OJ L 50, 27.2.2010, p. 8-12.

[9] Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. OJ L 285, 1.11.2003, p. 33-37.

Aflatoxins contamination is a global phenomenon, but generally crops in tropical and subtropical areas are more prone to contamination than those in temperate regions (Fandialan and Ilag, 1973; Hell et al., 2003; Widstrom, 1996). As climate plays a crucial part in the conditions that enhance AFs production, the problem also varies in severity from year to year. In Europe, AFs are considered as an "imported" problem, and therefore a strict control system is applied to the imported critical foods, like peanuts, pistachio nuts and maize (Curtui et al., 2004). Nevertheless, cereals contamination in Europe is revised in the following paragraph.

1.2.1. European surveys on aflatoxins occurrence in cereals

All European countries, those in the southern part included, normally are not characterised by warm and dry weather during cereals cultivation. In certain areas and years it happens and A. section Flavi can found suitable conditions for their growth and AFs production.

Surveys carried out to quantify AFs contamination in cereals in Europe showed that maize grain was the most investigated commodity followed by wheat. However, no surveys are available for AFs contamination in rice produced in Europe (Table 2).

In particular, in Italy 3607 maize samples have been analysed between 1982 and 2007 (AA. VV., 2005; Battilani et al., 2008a, b; Cinti et al., 2005; Decastelli et al., 2005; Micco et al., 1986; Pietri et al., 2004a, b; Piva et al., 2006; Reyneri, 2006) .

During these years, the maximum level (233 µg/kg) was found both in 2004 and in 2006 with mean values of 21 and 14 µg/kg, respectively (Battilani et al., 2008b). A high AFB_1 contamination (maximum level 155 µg/kg) was recorded in 2003 (Piva et al., 2006), a year characterised by very high temperatures during the whole growing season of maize and an extraordinary drought from early May to September.

Several surveys have been conducted in Turkey, a country with optimal climatic conditions for the activity of AFs producing fungi. A total of 423 maize samples have been considered for their AFB_1 contamination during the period 1986-2006 (Alperden et al., 1990; Alptekin et al., 2009; Giray et al., 2009; Gursoy and Bicici, 2003; Nizamlyodlu and Oguz, 2003; Oruc et al., 2006; Oruc et al., 2007; Ozay and Heperkan, 1989). The maximum level found was 432 µg/kg in 2006 (Oruc et al., 2007), even if also 2002 and 2005 have been characterised by high AFs contamination (maximum level 133 and 120 µg/kg, respectively) (Giray et al., 2009; Nizamlyodlu and Oguz, 2003). The maize sample with the highest level of AFB_1 contamination (431.90 µg/kg) (Oruc et al., 2007) was collected from a dairy farm in Bursa, where the dairy feed was prepared by the farmer from a maize grain that had been bought four months before. The level of 120 µg/kg (Giray et al., 2009) was found in a sample collected in the Mediterranean region of Turkey, characterised by warm climate suitable for the occurrence of mycotoxins. Instead, Nizamlýolu and Oguz (2003) explained that the level of 133 µg/kg and the high frequency of contamination might be related to the limited numbers of samples screened from poultry farm producers.

Romania is another EU country where extensive maize surveys have been carried out to quantify AFs contamination, even if with a more limited data set. Ninety-five maize samples have been analysed from 1997 to 2004 (Braicu et al., 2008; Curtui et al., 1998; Tabuc et al., 2009) with a maximum level of contamination of 46.4 µg/kg (Tabuc et al., 2009).

Surveys carried out on AFs contamination in wheat are less frequent and, consequently, available data are less complete than those for maize. However, a lower contamination in wheat than in maize has been detected. The higher AFs concentrations (6.4 µg/kg) have been found in Romania (Tabuc et al., 2009) where 106 wheat samples were analysed between 1997 and 2005 (Braicu et al., 2008; Curtui et al., 1998; Morar et al., 2007; Tabuc et al., 2009).

Table 2: Results of surveys for AFB_1 showing concentrations and distribution of contamination in maize and wheat in Europe

Country/Region	Year	Commodity	No of samples	LOQ+ (µg/kg)	n > LOQ	Mean (µg/kg)	Min/Max (µg/kg)	References
Southeastern Romania	2002/2004	Maize	54	5	20	6.86	0/46.4	(Tabuc et al., 2009)
Romania	2005	Maize	11	0.83	3		0/3.92	(Braicu et al., 2008)
Romania	1997	Maize	30	4*	0	-	-	(Curtui et al., 1998)
Italy	2002 (north Italy)	Maize	98	0.05*	na	0	-	(Battilani et al., 2008b)
	2003 (north Italy)	Maize	98			4.60	<0.05/155	
	2004 (Emilia Romagna)	Maize	84			16.6	<0.05/233	
	2004 (Emilia Romagna)	Maize	69			10.6	<0.05/233	
	2005 (Emilia Romagna)	Maize	237			2.3	<0.05/90	
	2006 (Emilia Romagna)	Maize	48			20	<0.05/233	
Italy	2004	Maize	84	0.05*	na	21	na	(Battilani et al., 2008a)
	2005	Maize	81			14	na	
	2006	Maize	39			14	na	
	2007	Maize	51			15	na	
Valle d'Aosta (Italy)	2004	Maize	28	5	1		>20	(Decastelli et al., 2005)
Italy	2003	Maize	110	0.05*	82	4.4	na/154.5	(Piva et al., 2006)
Northern Italy	1999	Maize	323	na	na		0/<50	(AA. VV., 2005)
	2000	Maize	360		na		0/<50	
	2003	Maize	441		na		0/>50	
	2004	Maize	344		na		0/>50	
Italy	2002	Maize	7	0.1	na	0.81	na	(Cinti et al., 2005)
	2003	Maize	1		na	35.2	-	
Italy (Lodi)	2002	Maize	10	0.05*	3	0.3	0/1.75	(Pietri et al., 2004a)
Northern Italy	1995	Maize	98	0.05*	na	1.9	<0.05/109	(Pietri et al., 2004b)
	1996	Maize	104		na	0.3	<0.05/13	
	1997	Maize	94		na	1.5	<0.05/32	
	1998	Maize	114		na	1.5	<0.05/28	

	1999	Maize	93		na	4.10	<0.05/158	
Italy (Piedmont)	2000	Maize	160	na	0	-	-	(Reyneri et al., 2003)
	2001	Maize	160		10	na	>0.2	
	2002	Maize	160		3	na	>0.2	
Italy (Piedmont, Lombardy and Veneto)	1982/1984	Maize	111	na	39	na	0.10/1.02	(Micco et al., 1986)
Switzerland	1987	Maize at harvest	33	0.2*	0	-	-	(Steiner et al., 1991)
	1987/1988	Stocked maize	22	0.2*	2	1.8	na/3.2	
Switzerland	1999	Maize	48	0.1*	10	0.7	0.1/1.5	(Noser et al., 2001)
	2000	Maize	51		5	0.4	0.2/0.9	
Turkey	1986	Maize	58	na	27		0/73.9	(Ozay and Heperkan, 1989)
Turkey	2002/2003	Maize	19	1*	na	10.94[a]	0.01/32.30[a]	(Oruc et al., 2006)
Turkey (Bursa region)	2006	Maize	5	1*	na	133	na/431.90	(Oruc et al., 2007)
Turkey (Kahramanmaras)	2005/2006	Maize	28	0.2*	21	14.90 (median=2.1)	nd/108.86	(Alptekin et al., 2009)
Turkey (Cukurova)		Maize	73	na	17	na	0.7/50[a]	(Gursoy and Bicici, 2003)
Turkey (Kinya)	2002	Maize	26	1.5*	15	na	<1.5/133	(Nizamlyodlu and Oguz, 2003)
Turkey		Maize	167	na	46 %	na	3/70	(Alperden et al., 1990)
Turkey	2005	Maize	47	1.75*	22	na	<1.75/120.33[a]	(Giray et al., 2009)
France	1998/1999	Maize imported in UK	97	0.2	na	na	<0.1/5.8	(Scudamore and Patel, 2000)
Croatia	1978/1979	Maize	na	na	4	na	5/50	(Pepeljnjak and Balzer, 1982)
Cyprus	1997	Maize	55	0.1*	0	-	-	(Ioannou-Kakouri et al., 2004)
	1998	Maize	65	0.1*	0	-	-	
	1999/2003	Maize	52	0.2*	0	-	-	
Czech Republic	2008	Maize	8	na	0	-	-	(Monbaliu et al., 2010)
Spain	2008	Maize	14	na	0	-	-	(Monbaliu et al., 2010)
Portugal	2008	Maize	12	na	0	-	-	(Monbaliu et al., 2010)
Spain (Galicia)	1983	Fresh maize	55	na	0	-	-	(Munoz et al., 1990)
Romania	2002/2004	Wheat	35	5	na	1.83	0/6.4	(Tabuc et al., 2009)
Romania	2005	Wheat	26	0.83	9		0/5.7	(Braicu et al., 2008)

Romania	1997	Wheat	25	4*	0	-	-	(Curtui et al., 1998)
Romania (Transylvania)	2005	Wheat	20	na	3		0/6	(Morar et al., 2007)
Cyprus	1997	Wheat	27	0.1*	0	-	-	(Ioannou-Kakouri et al., 2004)
	1998	Wheat	3	0.1*	0	-	-	
	1999/2003	Wheat	30	0.2*	0	-	-	
Turkey	2002/2003	Wheat	41	0.1*	17	na	<0.01/0.14	(Giray et al., 2007)
Turkey (Cukurova)		Wheat	43	na	7	na	0.18/3.5[a]	(Gursoy and Bicici, 2003)
Italy (Sicilia)	2005	Wheat	165	0.5*		1.0	na/1.7	(Gallo et al., 2008)
	2006		203	0.5*		1.2	na/2.7	
Czech Republic	2008	Wheat	8	na	0	-	-	(Monbaliu et al., 2010)
Denmark	2008	Wheat	14	na	0	-	-	(Monbaliu et al., 2010)
Hungary	2008	Wheat	7	na	0	-	-	(Monbaliu et al., 2010)
Algeria	2004 (Mitidja region)	Wheat	3	0.005 *	2	-	-/3.41	(Riba et al., 2010)
	2006 (Mitidja region)	Wheat	13		8	-	-/13.45	
	2004 (Setif region)	Wheat	2		1	-	0.87	
	2006 (Setif region)	Wheat	10		6	-	-/7.0	

(+): LOQ, limit of quantification (this information is not always available; some studies report the limit of detection)
(*): LOD: limit of detection
(na): not available
(nd): not detected
([a]): total aflatoxins
Grey lines: studies with georeferenced data

Data presented in Table 2 show that EU AFs contamination is a problem concerning especially maize grain. In particular, EU countries with the more representative maize contamination are Italy and Romania. Among EU countries, the maximum level (233 µg/kg) was found in Italy, even if the highest maize contamination reported was in Turkey (431.9 µg/kg). Aflatoxins presence in wheat is not a main concern in most of the countries, but it is of interests in Romania.

Some studies with incomplete data are also available; these data are not reported because they lack the geographic position of sampling that is crucial for the aim of this study.

A 2-year (October 2003-September 2005) survey program was initiated by feed additive producer Biomin® in order to evaluate the incidence of mycotoxins in feed and feed raw materials in some of the major animal production (Binder et al., 2007). The authors give an overview of the numbers of analytical tests performed on samples with regard to their sourcing origin (Northern, Central and Southern European and Mediterranean countries), with also the respective arithmetic mean, median and maximum levels detected. They also give an overview of contamination of commodities tested, stating the total number of each commodity tested, the number of positives, arithmetic mean and median of positive samples as well as the maximum level identified per commodity.

More data are available in studies where the sampling years or place are not specified (Alp et al., 1997; EFSA, 2007; Heperkan, 2006; Malmauret et al., 2002; Scudamore et al., 1997; Sedmikova et al., 2001; Simion et al., 2008).

Some of the cited studies describe the distribution of positive samples and the ranges of AFB_1 levels according to the sampled place (Alptekin et al., 2009; Battilani et al., 2008a, b; Braicu et al., 2008; Gallo et al., 2008; Giray et al., 2009; Morar et al., 2007; Ozay and Heperkan, 1989). These georeferenced data are very important for model validation.

From this data collection, it emerges that in Europe there are only few studies about AFs contamination in cereals that provide complete information (kind of commodity, sampling year and country, sample number, mean concentration, etc.). Most exhaustive data have been found about AFs contamination in maize in Italy and Turkey and in wheat in Romania, where climatic conditions are sometimes favourable to AFs producer fungi growth on that specific commodity.

No data are available about AFs contamination on rice cultivated in Europe; therefore surveys carried out in other parts of the world, where rice cultivation is more extensive and climatic conditions are more favourable to AFs production, were surveyed and only 1 paper was found.

Hussaini et al. (2007) analysed 28 visibly mouldy samples of rice in Niger State of Nigeria, an area with favourable climate for A. section *flavi* growth and mycotoxins production. The average annual temperature is 31.7 °C and the average humidity is 51.6 %; it is warm and humid, especially between May and October (29.5 °C and 73.1 %) The field samples have been taken during the dry harmattan season, shortly before harvest period in 2000. Eleven out of twenty eight samples analyzed were AFB_1 positive ranging from 0 to 464 µg/kg, with a mean concentration of 35.89 µg/kg.

Some papers report the identification and the incidence of *Aspergillus* spp. colonising rice grains in South Asia (Reddy et al., 2010a), Brazil (de Carvalho et al., 2010; Guimarães et al., 2010) and Nigeria (Somorin and Bankole, 2010); the detection of different mycotoxigenic fungi, including *Aspergillus* spp., regarded also stored rice in Africa (Surekha et al., 2011). Among these reports, only in Brazil low levels of aflatoxin contamination were reported (de Carvalho et al., 2010) and different Indian rice cultivars have been studied for the accumulation of AFB_1 after in vitro inoculation with *A. flavus* (Reddy et al., 2010b).

The notifications of RASFF suggest the relevant countries where data could be found. These data could be useful to validate the predictive models, but only one paper is available. The attention to food and feed safety is limited in those countries where the risk for AFS contamination is high; probably for that reason data available are very limited.

1.3. The crops

1.3.1. European distribution of maize, wheat and rice

Data on European distribution of selected crops were extracted from FAOSTAT, a database managed by FAO and available at http://faostat.fao.org/site/291/default.aspx. FAOSTAT provides access to over 3 million time-series and cross sectional data relating to food and agriculture; it contains a full matrix of integrated and compatible statistics coverage of 200 countries, 15 years, and more than 200 primary products and input items related to production, trade, resources, consumption and prices.

Maps were drawn with all European countries shared in 3 groups based on the country surface grown with a specific crop (Figures 2, 3, 4).

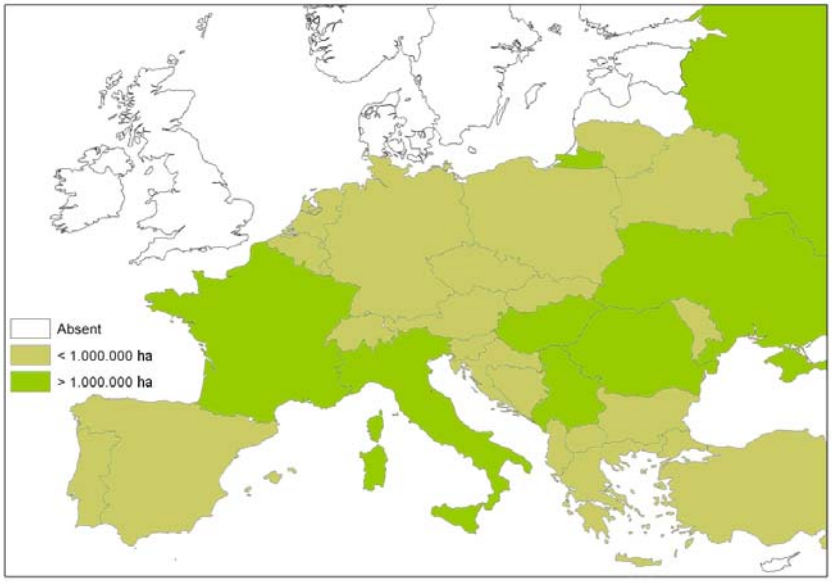

Figure 2: European distribution of maize crops

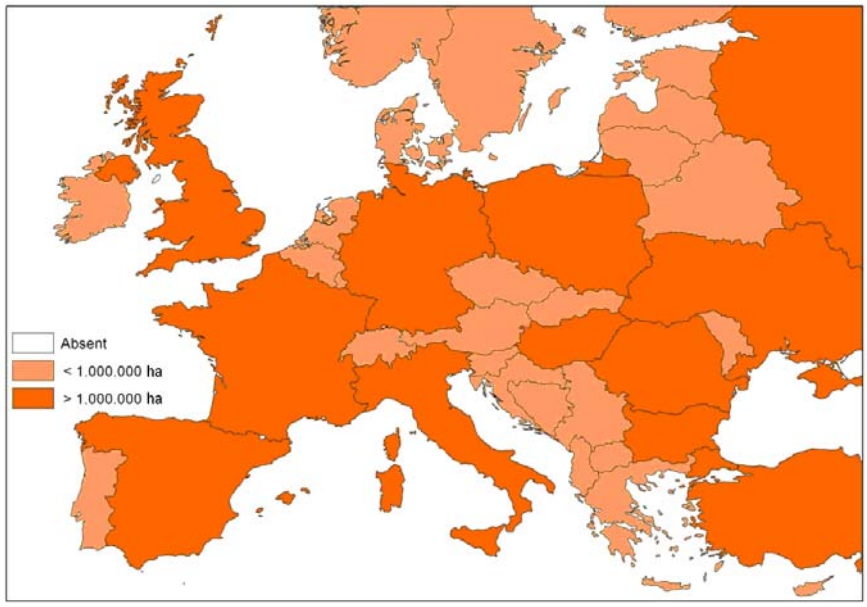

Figure 3: European distribution of wheat crops

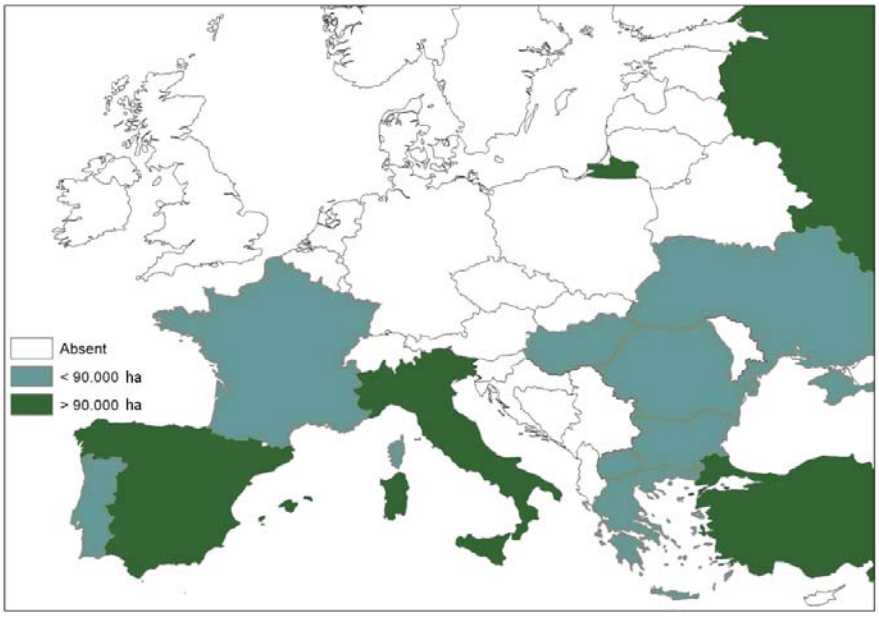

Figure 4: European distribution of rice crops

Data on EU distribution and production of selected crops were extracted from Eurostat (http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_cpp_crop&lang=en). Eurostat is the Statistical Office of the EC. Its mission is to provide the EU with high-quality statistical information. For that purpose, it gathers and analyses figures from the national statistical offices across Europe and provides comparable and harmonised data for the EU to use in the definition, implementation and analysis of Community policies.

The most recent data are presented here (reference year 2009; Table 3) showing the situation in the 27 EU Member States (EU-27), and in other European countries.

Wheat is the most important cereal crop, in terms of cultivation area, with 25,615,600 ha in EU (27 countries). The wider wheat growing area is in France (5,146,600 ha), followed by Germany (3,226,000), Poland (2,346,200), Romania (2,148,800), Italy (1,795,500) and Spain (1,772,500).

Maize is also an important crop for Europe, with a growing area of 8,362,800 ha in EU (27 countries) and a mean production of per ha. Romania (2,338,900 ha), France (1,679,800), Hungary (1,177,300) and Italy (915,500) contribute with major surface, even if mean production is very variable among countries.

Maize is a crucial crop for Europe, with about 14 million ha grown for different purposes such as grain for food, feed and processing, green maize for silage or biogas production. A study was organized to collect information regarding maize-based crop systems located in four European regions significantly different in term of pedo-climatic and socio-economic situations (Vasileiadis et al., 2011) defined as follows:

(i) Northern regions, with Denmark and the Netherlands;

(ii) Central-eastern regions, with Tolna and Békés countries in Hungary;

(iii) South-Western regions, with Ebro Valley in Spain;

(iv) Southern regions, with the Po Valley in Italy;

A further detail was added, related to the type of maize production (silage/grain), cropping sequence (crop rotation/continuous maize) and irrigation (irrigated/not irrigated).

Silage maize dominated in the northern region, continuous or rotated, but not irrigated. In Hungary, grain maize not irrigated was found as largely dominant, continuous in Tolna and in rotation in Békés. In Ebro valley in Spain, the main systems reported were grain or silage maize in rotation and irrigated (83 % of total maize growing area), but some continuous grain maize was also stated. In the southern region, the Po Valley, grain and silage maize irrigated cover 80 % of the growing area, 50 % in rotation and 30 % continuous; grain maize in rotation and without irrigation contribute to the remaining 20 %.

Rice: although EU is only 17th among the main world producers of rice, periodic production estimates at EU-27 level are of interest for the EC. The rice growing area within the EU is about 450,000 ha: 238,000 ha in Italy, 119,000 ha in Spain, 28,000 ha in Portugal, 29,000 ha in Greece, 29,000 in France, 24,300 ha in Romania, 13,300 ha in the Former Yugoslav Republic of Macedonia and 3,100 ha in Hungary. Smaller rice-cropped areas are located also in Eastern Europe.

During the last 10 years rice cultivation in the EU has remained roughly unchanged. The two top rice producers are Italy and Spain. These two countries together contribute more than 80 % of the total rice production in Europe. Some slight variations in the harvested area have been recorded in each country of cultivation, in relation to the market price or water availability. In most countries, rice production mostly occurs in limited areas such as, the Po valley in Italy, the Rhone delta in France, the Thessaloniki area in Greece. In Spain and Portugal rice cultivation is scattered in several areas such as the Aragon area, the Ebro delta, the Valencia Albufera, the Guadalquivir valley in Spain, the Tejo and Mondego valleys in Portugal.

The ecological conditions of rice cultivation are quite variable. In Italy, the climate of rice production is temperate-continental, with a cold winter and warm summer and main rainfall occurring during the

first stages of the crop growth (April-June) and the harvesting period (September-October) (FAO, 1996). In most of the other countries the climate is sub-tropical (Mediterranean climate) with a dry summer and warm, dry, clear days and long growing season.

Table 3: Data of EU area of production and yields of selected crops in 2009 from Eurostat (updated with data from FAOStat, 1 August 2011)

Country	Grain maize		Wheat		Rice	
	Area of production (1000 ha)	Yields (100 kg/ha)	Area of production (1000 ha)	Yields (100 kg/ha)	Area of production (1000 ha)	Yields (100 kg/ha)
27 EU countries						
European Union (27)	8362.8	1504.5	25.615.6	1304.9	463.1	410.2
Austria	178.5	105.9	309.0	49.3	na	na
Belgium	66.7	121.2	211.5	93.5	0.0	na
Bulgaria	274.2	47.1	1247.7	31.9	8.3	52.3
Cyprus	na	na	5.8	25.5	na	na
Czech Republic	105.3	84.5	831.3	52.4	0.0	na
Denmark	0.0	na	739.0	80.4	0.0	na
Estonia	0.0	na	113.6	30.1	na	na
Finland	na	na	218.3	40.6	na	na
France	1679.8	91.1	5146.6	74.5	24.2	57.1
Germany	464.3	97.5	3226.0	78.1	na	na
Greece	240.0	98.0	698.0	26.2	29.0	70.7
Hungary	1177.3	63.9	1146.5	38.5	2.7	43.2
Ireland	na	na	84.5	81.7	0.0	na
Italy	915.5	86.1	1795.5	35.3	238.5	na
Latvia	na	na	285.7	36.3	na	na
Lithuania	5.5	43.3	500.0	42.0	na	na
Luxembourg	0.4	60.0	13.8	65.7	na	na
Malta	na	na	na	Na	na	na
Netherlands	18.8	130.0	150.9	92.9	0.0	na
Poland	274.1	62.3	2346.2	41.7	na	na
Portugal	97.0	65.1	60.8	16.8	27.9	55.9
Romania	2338.9	34.1	2148.8	24.2	13.3	54.3
Slovenia	38.6	78.4	34.3	39.8	na	na
Slovakia	139.0	71.1	380.3	40.4	na	na
Spain	347.6	100.6	1772.5	26.9	119.2	76.7
Sweden	1.3	64.3	374.0	60.9	na	na
United Kingdom	na	na	1775.0	79.3	na	na
Other European countries						
Albania	na	na	na	na	na	na
Bosnia & Herzegovina	188.7	51.0	67.8	37.8	na	na
Croatia	296.9	73.5	180.4	51.9	na	na
Former Yugoslavic Republic of Macedonia	30.8	40.0	86.0	31.5	3.1	56.0
Iceland	na	na	na	na	0	na
Liechtenstein	na	na	na	na	0	na
Norway	na	na	na	na	0	na
Switzerland	16.7	104.1	91.3	60.2	0	na
Turkey	na	na	na	na	na	na

(na): data not available

1.3.2. Cereal Crop Growth Models

Cereals, mainly maize and wheat, are major crops in Europe. They are basic commodities used for the production of both animal feed and human food products. Many different types and varieties of wheat and rice and maize hybrids are grown all over Europe. Although cereals cultivation systems are of a high technical level, the quality and safety of grain and derived products can be reduced due to

contamination by mycotoxins. Fungi that produce these toxins are growing on the cereal plants and ears during the cultivation season and on the harvested grain during storage and even during processing. Fungi may infect cereals in all growth stages, from sowing up to harvest, but some growth stages and parts of the plant are more vulnerable to infection by mycotoxin producing fungi than others. Both crop and fungal disease development depend on environmental conditions; hence they are likely to be influenced by climate changes. Understanding the development and growth of both cereal plants and pathogenic fungi during the cultivation period, the factors involved, as well as their interaction, is important to predict and manage risks for mycotoxin contamination, mainly in a climate change scenario.

Crop growth models are an essential part to predict high-risk conditions for fungal infection and toxin formation. Crop growth models in general consider all the factors that affect the physiology and development of the plants (Brisson et al., 2003). For all crops, growth and development is a process where water, CO_2 and nitrogen are transformed into biomass using sun light as energy source. As in all organisms, crops grow and develop faster when temperatures are higher, but there are lower and upper limits for growth and survival.

Cereals growth stages can be described in 10 different steps listed as: 1. germination, 2. leaf development, 3. tillering, 4. stem elongation, 5. booting, 6. inflorescence emergence or heading, 7. flowering and anthesis, 8. development of fruits, 9. ripening and 10. senescence well described in the scale known as BBCH (Lancashire et al., 1991). The first 5 steps relate to the vegetative stage and the following to the productive one; grains are normally harvested at ripening and only wild crops stay in field till to senescence.

The development stage of a plant defines its physiological age and is characterized by the formation of the various organs and their appearance. The most important phenological change is from the vegetative to the reproductive stage, determining the change in dry matter allocation over organs. As many physiological and morphological processes change with the phenological stage of the plant, accurate quantification of phenological development is essential in any simulation model for plant growth. The crucial growth stage for cereal crops is flowering because any problem in this period has strong effect on yield; this is also a crucial step for the interaction with mycotoxin producing fungi that start their infection cycle on host plants at flowering.

1.3.3. Maize growth models

1.3.3.1. Maize hybrids and season length

Maize crops in Europe are all seeded with commercial hybrids while varieties are no more used. The hybrids are the result of crossing 2 inbreed lines and have the advantage of giving the best productivity, due to the heterosis, and the highest homogeneity of plants.

The hybrids classification is based on their season length, intended as the number of days requested from germination to ripening; short season hybrids need a minimum of 110 days while 135 days are necessary for long season hybrids. They are also ranked in FAO classes and those used in Europe belong to classes from 200 to 700, being the former short season and the latter those with a longer growing season; longer seasons allow higher production.

Maize can be grown for different usages and harvest can be planned at different stages of crop maturity. Most of maize is grown for grain production and this is also that of concern for mycotoxin contamination, mainly for AFs. Therefore, grain maize is considered in this report.

Maize is seeded during spring, when the minimum temperature for the crop (10 °C) is common in field, that means between late March and May, depending on the European growing area. Short

season hybrids are grown in the northern areas of maize cultivation or as second crop in southern Europe. Hybrids with a season length between 125 and 135 days are preferred in South Europe, but FAO class 300 hybrids are commonly seeded in southern Europe as not irrigated crops.

1.3.3.2. Maize phenology

Maize plants have separate male and female inflorescence, with the former, held at the top of the plant, emerging earlier than the latter, normally visible at the mid height of the plant. Several female flowers can be present on maize plants, but breeding selected plants with only 1 female ear. Maize growth stages are well described in Figure 5; however, a BBCH scale is usually used to explain the exact development stage of maize (Table 4).

The crucial growth stages are 6 and 8, flowering and ripening respectively. In particular, growth stage 67, described as male flowering completed and stigmata drying in female inflorescence is that commonly defined as "flowering". Growth stage 89, defined as fully ripe, with kernels hard and shiny, about 65 % dry matter, is that intended as ripening, even if the crop is normally harvested with humidity included between 14 % and 30 %, depending on the season length of the hybrid and the weather conditions around the ripening period.

Grain drying and ventilation is always suggested post-harvest to have homogeneous humidity around 13-14 % in the whole mass to be stored. Drying must be managed as soon as possible after harvest, in any case before 36-48 hours, in order to avoid fungal growth and mycotoxin production, being humidity and temperature commonly suitable for several pathogens, mainly *A.* section *Flavi*. Drying must be managed carefully, not too quickly and with not too high temperatures, to avoid kernels cracks, a possible entry way for fungi.

Maize is stored for long periods, until the production of the following year is available, but it can be preserved for more years.

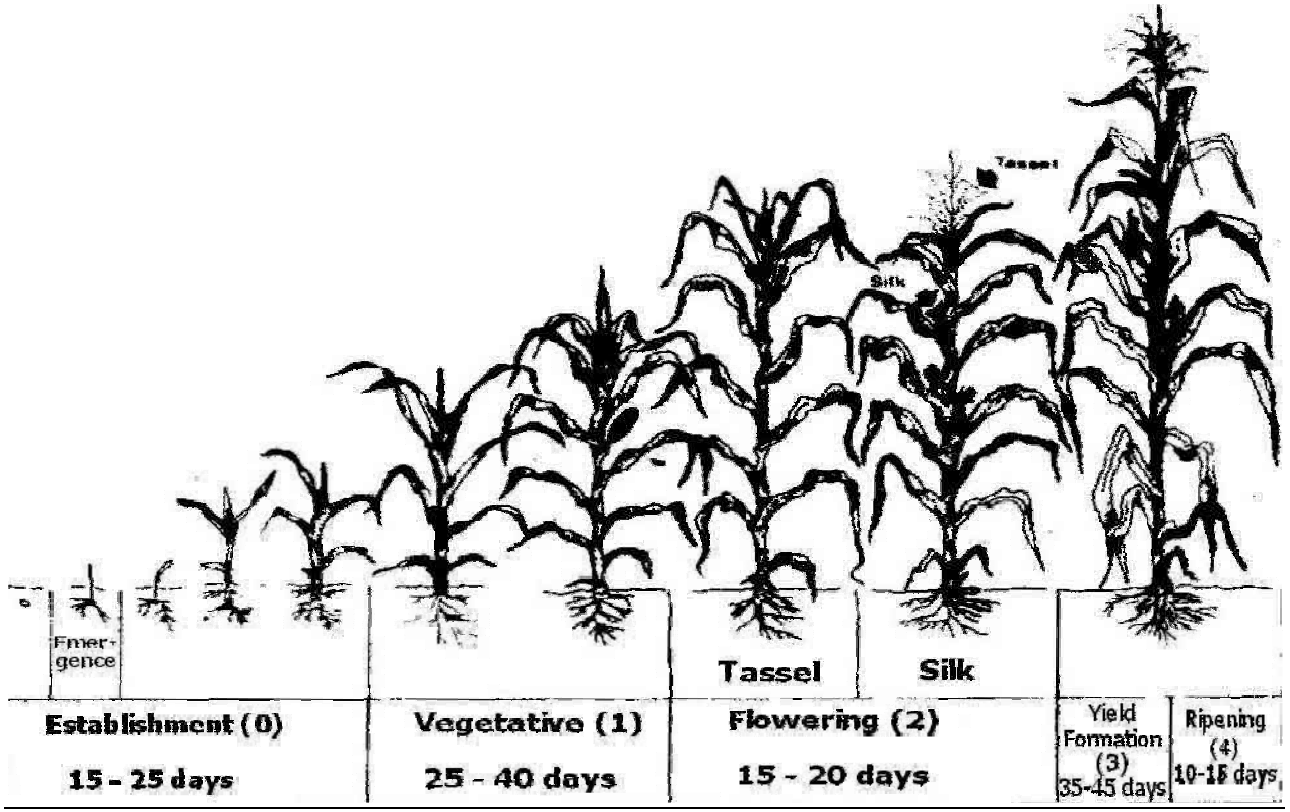

Figure 5: Maize growth stages (FAO, on line)

Table 4: Phenological growth stages and BBCH-identification keys of maize (Weber and Bleiholder, 1990; Lancashire et al., 1991), see also http://www.jki.bund.de/fileadmin/dam_uploads/_veroeff/bbch/BBCH-Skala_englisch.pdf

Code	Description
Principal growth stage 0: Germination	
00	Dry seed (caryopsis)
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle emerged from caryopsis
06	Radicle elongated, root hairs and /or side roots visible
07	Coleptile emerged from caryopsis
09	Emergence: coleoptile penetrates soil surface (cracking stage)
Principal growth stage 1: Leaf development 1, 2	
10	First leaf through coleoptile
11	First leaf unfolded
12	2 leaves unfolded
13	3 leaves unfolded
1* .	Stages continuous till . . .
19	9 or more leaves unfolded
Principal growth stage 3: Stem elongation	
30	Beginning of stem elongation
31	First node detectable
32	2 nodes detectable
33	3 nodes detectable
3* .	Stages continuous till . . .
39	9 or more nodes detectable3
Principal growth stage 5: Inflorescence emergence, heading	
51	Beginning of tassel emergence: tassel detectable at top of stem
53	Tip of tassel visible
55	Middle of tassel emergence: middle of tassel begins to separate
59	End of tassel emergence: tassel fully emerged and separated
Principal growth stage 6: Flowering, anthesis	
61	Male: stamens in middle of tassel visible; Female: tip of ear emerging from leaf sheath
63	Male: beginning of pollen shedding; Female: tips of stigmata visible
65	Male: upper and lower parts of tassel in flower; Female: stigmata fully emerged
67	Male: flowering completed; Female: stigmata drying
69	End of flowering: stigmata completely dry
Principal growth stage 7: Development of fruit	
71	Beginning of grain development: kernels at blister stage, about 16 % dry matter
73	Early milk
75	Kernels in middle of cob yellowish-white (variety-dependent), content milky, about 40 % dry matter
79	Nearly all kernels have reached final size
Principal growth stage 8: Ripening	
83	Early dough: kernel content soft, about 45 % dry matter
85	Dough stage: kernels yellowish to yellow (variety dependent), about 55 % dry matter
87	Physiological maturity: black dot/layer visible at base of kernels, about 60 % dry matter
89	Fully ripe: kernels hard and shiny, about 65 % dry matter
Principal growth stage 9: Senescence	

97	Plant dead and collapsing
99	Harvested product

(*): the second number increases with the increase of leaves or nodes number

1.3.3.3. Maize models

Modelling plant development, intended as the series of processes related to cell differentiation, organ initiation, organ appearance and extends to plant senescence (Streck et al., 2003), is an important tool, useful for growers and included in many crop simulation models (Costa and Barros, 2001). Accurate prediction of crop development is also very important in studies related to climate change scenarios (Streck and Alberto, 2006).

Temperature, in particular air temperature, is considered the main driving variable for maize development (Coelho and Dale, 1980; Cutforth and Shahykewich, 1990; Daughtry et al., 1984; Warrington and Kanemasu, 1983). Several models have been developed to simulate maize phenology and they can be grouped in linear (Daynard, 1972; Gilmore and Rogers Junior, 1958; Major et al., 1983) and non-linear models (Coelho and Dale, 1980; Cutforth and Shahykewich, 1990; Daughtry et al., 1984; Warrington and Kanemasu, 1983) .

Linear models represent the simpler approach; the thermal time is calculated with the addition, on a daily base, of degrees day, or degrees exceeding the base temperature, intended as the minimum temperature useful for the crop (Gilmore and Rogers Junior, 1958). An improvement of this simple approach is to consider an upper threshold temperature and a linear decrease in the accumulated thermal time beyond an optimum value down to zero, at the maximum temperature (Streck and Alberto, 2006; Streck et al., 2007). Thermal time is often a better descriptor of growth stages than calendar days (days after crop planting or emergence, day of the year) (Gilmore and Rogers Junior, 1958; McMaster and Smika, 1988; Russelle et al., 1984), but not always (Sentelhas and Ungaro, 1998; Yuan and Bland, 2005).

The minimum temperature generally accepted for maize is 10 °C and the thermal time requested for BBCH 67 ranges between 620°d and 870°d for hybrids belonging to FAO class 200 and 700 respectively. Ripening (BBCH 89) is reached with 1250-1500°d respectively in FAO class 200 and 700 respectively.

The assumption of a linear relationship between temperature and development can be criticised because it is not completely rational from a biological point of view, while a non linear response is more reliable (Bonhomme, 2000; Wang and Engel, 1998). In fact, non linear models provided better prediction than linear models in different crops, like wheat (Schroder and Sondgerath, 1996) and maize (Cutforth and Shahykewich, 1990) included.

An interesting model has been developed by Wang and Engel (1998). The WE model simulates crop development based on the non linear effect of environmental factors represented by response functions that range between 0 and 1. The temperature rate is described by a beta function, which has 3 coefficients with biological meaning: minimum, optimum and maximum temperature for development. The WE model has been originally developed for wheat, it was used for other annual crops (Streck et al., 2007), maize included (Streck et al., 2008b). In maize, it gave excellent prediction of silking in all sowing dates with differences between predicted and observed days of silking between 0 and 5; good results have been obtained also for physiological maturity with differences included between -7 and 0 days. Further models have been developed to predict maize growth, development and yield, with more input variables requested; some examples follow (Table 5).

Reliable prediction of maize phenology has been obtained by Zheng and Gao (2000) taking into account temperature and day length; the relative error was less than 5 % when predicted data were compared with filed surveys.

Temperature, solar radiation, soil water and soil nitrogen were considered by Bonato et al. (1999) to develop a population model for maize growth and development. The effect of nitrogen concentrations on maize phenology have been well described by the model; it provided a satisfactory fit to field data from maize grown under different planting densities and the structure of the model permits the evaluation of many concomitantly operating stress factors.

Hybrid-maize is a simulation model developed by combining 2 modelling approaches: the growth and development functions of CERES-maize (Jones, 1986; Jones et al., 2003) and the mechanistic formulation of photosynthesis and respiration in generic crop models such as WOFOST (van Ittersum et al., 2003b). The aims, which is to predict leaf area, dry matter accumulation and final yield have been successfully gained, but further improvements were proposed by the authors for a more profitable use in practice (Yang et al., 2004a).

Specific researches were also developed to collect data useful for the improvement of existing models. In fact, it is stressed that any factor involved in the cropping system can influence the schedule of growth stages and the final crop yield.

Fletcher and Moot (2002) and Gungula (2003) studied the effect of sowing date and fertilizers on sweet maize phenology. They found a relevant role of phosphorus, together with nitrogen, and they quantified crop response in term of phenology.

The role of water, in terms of irrigation and rainfall, has been considered respectively by Farre et al. (2000) and by O'Neal et al. (2002) and they quantified the effect on crop phenology and yield.

As a general comment, the prediction of maize growth stages was approached by several authors (several models) and air temperature was accepted as the most relevant driving variable. Different approaches can be followed to relate temperature and crop phenology: a simple linear relationship or a non linear one, both, showed a good agreement with observed data. Considering the aim of this project, even if other studied variables can influence growth stages, it is more realistic to think about the temperature based model because it is applicable with climate change scenario that needs to be generated.

The WE Model seems the most promising.

Table 5: Papers published on models development and influential factors for the prediction of cereals growth stages

Crop	Authors	Model name	Temperature	Solar radiation	Soil water	Day length	Sowing date	Nitrogen	Phosphorus	Photo synthesis	Respiration	Genotype *
Maize	(Bonato et al., 1999)		x	x	x			x				
Maize	(Farre et al., 2000)			x	x							
Maize	(Fletcher and Moot, 2002)		x					x	x			
Maize	(Gungula et al., 2003)	CERES						x				
Maize	(Zheng and Gao, 2000)		x			x						
Maize	(Streck et al., 2008a)	WE	x									
Wheat	(Wang and Leuning, 1998)	WE	x			x	x					
Maize	(Yang et al., 2004b)	Hybrid-Maize	x							x	x	x
Wheat	(van Bussel et al., 2010)	AFRCWHEAT2	x			x	x					
Wheat	(Saiyed et al., 2009)		x			x	x					
Wheat	(Ewert et al., 1996)	AFRCWHEAT2	x			x	x					
Wheat	(McMaster et al., 2008)	CERES	x			x	x					
Wheat	(Harrison et al., 2000)	AFRCWHEAT2	x			x	x					
Rice	(Van Keulen et al., 1982)	SUCROS	x	x							x	
Rice	(Van Keulen and Wolf, 1986)	WOFOST	x	x							x	
Rice	(Jones et al., 1984)	CERES		x								
Rice	(Keating et al., 2003)	APSIM	x		x			x	x			
Rice	(Casanova et al., 2000)	ORYZA1	x									

Rice	(Confalonieri et al., 2009a)	WARM	x	x			x	
	(Tang et al., 2009)	RiceGrow	x			x		
	(Krishnan et al., 2007)	INFOCROP	x				x	
	(Maruyama and Kuwagata, 2008)		x	x				x

(*): Genotype is the generic term for both hybrids (maize) and cultivars (wheat and rice).

1.3.4. Wheat growth models

1.3.4.1. Wheat varieties and season length

Wheat crops are generally categorised as winter wheat, which is sown in late autumn, or spring wheat, which is sown in early spring. Winter wheat flowers earlier and is harvested earlier in the year than spring wheat. Crop growth, flowering time and number of days needed for ripening of the grain depend primarily on temperature and, to a lesser extent, also on day length. Wheat breeders have developed different crop varieties which are suitable for different areas and climatic conditions in Europe. They deliver so called early and late varieties that have different growing characteristics and vary with the time to flowering and harvest in order to optimize yields. Crop growth models mostly focus on average growing patterns but cultivar variability can have major impact on model outcomes. Crop growth models normally aim at predicting yield. Wheat yields are determined to a large extent by the length of the growing season and timing of the various developmental stages. Therefore phenology has always to be included in yield predicting models (Ewert et al., 1996; Jamieson et al., 1998). Hence these models often can also be used to predicting the growth stages that are sensitive to fungal infection in wheat.

1.3.4.2. Wheat models

In wheat, as well as in other crops, the growth and development increases proportionally with rising temperature in most of the normal temperature range. However, growth is retarded at very high temperatures and extreme temperatures may have severe negative impacts on growth and development (Porter and Gawith, 1999). Depending on developmental stage and soil water levels such negative impacts occur above 25 ^{o}C early in the season to above approximately 30-35 ^{o}C at the end of the growing period. In winter, wheat cold hardiness is available down to approximately -17 ^{o}C.

Based on the foregoing, most advanced wheat production models use water availability, CO_2 levels, nutrients, solar radiation and temperature as input factors to predict the development and growing of the crop. As water and nutrients are often considered as unlimited and CO_2 levels impacts are often considered to be negligible, most common models are driven by temperature and day-length patterns (Brisson et al., 2003; van Ittersum et al., 2003b). Wheat crop research has traditionally focused on optimising yield for each particular wheat type, and is primarily economically driven. This is done by breeding and optimising crop management systems. As part of this, models are used as a tool for understanding and predicting wheat development and growth. The phenology of the crops, i.e., the sequence of growth stages from seed, seed emergence, leaf formation, heading, flowering and ripening of the grain kernels, has always been studied as an integral part of the process for obtaining high grain yields and good quality (Carver, 2009).

For the fungal infections, in particular the phenological sequence of growth stages during the season is important. Although advanced crop growth models, such as reviewed by Lenz-Wiedemann (2009), often include phenological aspects and include all wheat growth factors, they are often too complex to be linked to predictive models of fungal infection and mycotoxin production. Simple models that focus on phenology are more relevant in this context. Such models are often primarily driven by temperature. However, the impact of other factors should be known as well to ensure the models will have accurate predictions and can be used over large areas, with different weather conditions and under future climate scenarios.

1.3.4.3. Temperature and wheat phenology

In wheat, phenology is modelled as a sequence of growth stages (GS) which are most commonly described according to the system of Zadoks (1974) as shown in Figure 6.

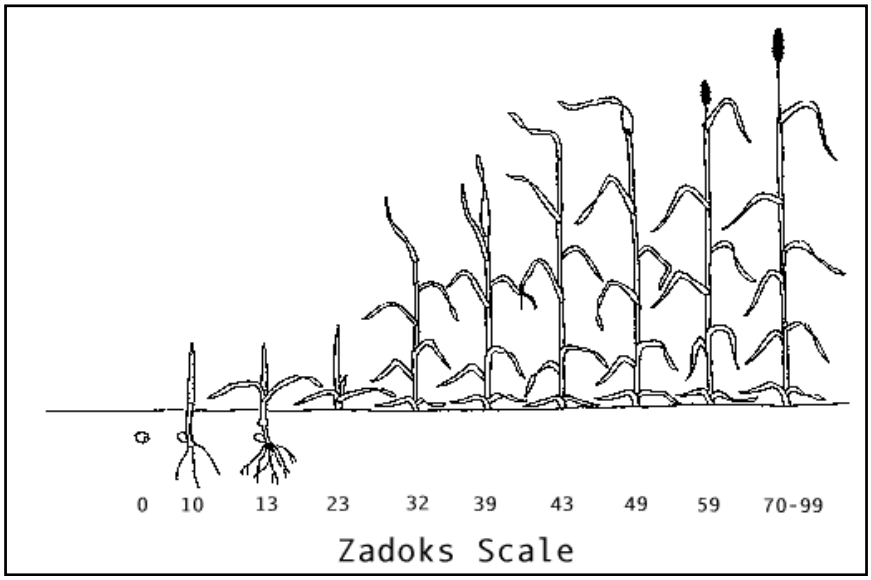

Figure 6: Classification of the growth stages of wheat according the Zadoks scale

The heading and flowering periods (GS 50 to GS 69) are considered to be most critical for fungal infections. The actual calendar dates and the start of heading and flowering (anthesis) and the time to harvest primarily depend on temperature and genetic characteristic of the crop (Ewert et al., 1996).

Models for these temperature-phenology relations have been developed and elaborated upon since more than 50 years and are still being improved and modified. These models have extensively been reviewed by Saiyed (2009). The most widely applied models in Europe and elsewhere are AFRCWHEAT2 (Ewert et al., 1996), WOFOST (Van Diepen et al., 1989), CERES/DSSAT (Jones et al., 2003) of which the first is more focused on wheat, the other being more general.

Basically there are 2 types of models, i.e., temperature sum models and simulation models. The *first type* is based on the idea that a fixed amount of *heat units* is needed for each wheat developmental stage (see Figure 6), and that by summing up daily temperatures from sowing to the start of flowering and the time of harvest can be calculated.

The *second type* uses experimentally established *relationships between temperature and developmental rate* and uses daily temperature data to simulate the development of crops over time (van Ittersum et al., 2003a). In principle, the approaches are similar but the first type is usually based on field observations over years, whereas the second type is mostly based on indoor experiments. Many publications present developmental rate for each stage as the inverse of the temperature sum required for development for that stage. An important principle is that each growing stage has its characteristic (cultivar dependent) heat sum requirement (Saiyed et al., 2009).

By comparing the total temperature sum required from seeding to harvest (Figure 7) an indication is available of climatic regions that are suitable for the growing of particular crops. If the required temperature is not reached it will be impossible to grow the crop or crop cultivar anyway (Harrison et al., 2000).

Temperature-sums or thermal time requirement are expressed as the number of degree-days needed to complete one or more growing stages usually starting from the sowing date. The temperature (as degrees above a certain minimum) is simply accumulated over periods from one stage to another (i.e., from sowing to leaf emergence, from emergence until flowering, or from flowering to harvesting).

Several methods are available to calculate temperature sums for each growing stage according to characteristic plant responses (mostly including a base temperature and an optimum temperature) (Ewert et al., 1996; Saiyed et al., 2009).

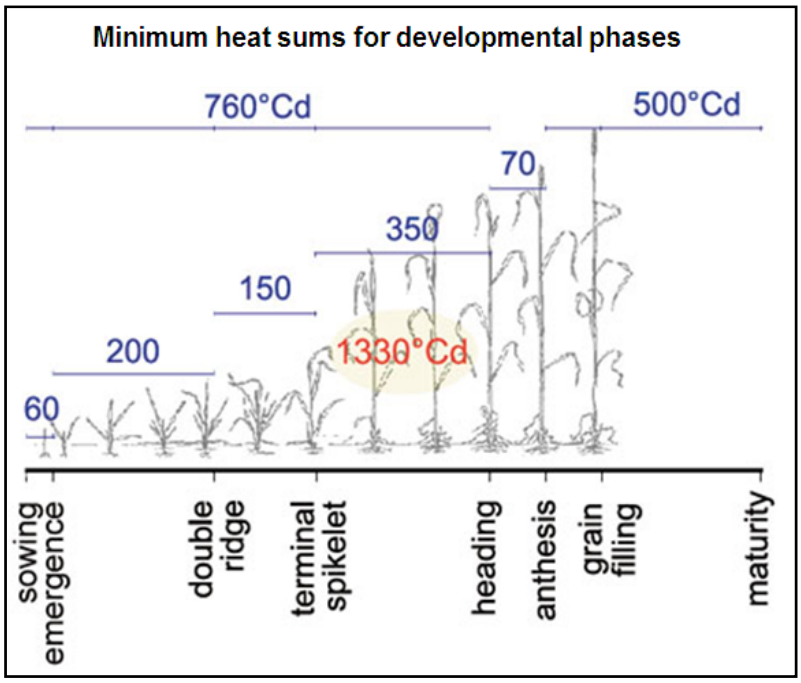

Figure 7: Example of thermal time requirements (degree-days) for the different developmental stages in wheat (copy from www.fao.org). (°celsius: degree centigrade per day)

For each particular plant species or crop type (of a defined cultivar), the required number of heat-units (degree-days) to reach a particular phenological phase appears to be rather constant over years with different weather conditions. Therefore, the start of flowering and harvest of wheat can satisfactory be predicted by temperature sums starting from the sowing date. In simulation models that mimic growth and development in time the underlying temperature-growth rates relationship is often used and other growth limiting factors such as water stress and nutrient levels can be included (Craufurd and Wheeler, 2009; Jamieson et al., 1995; Matsuoka et al., 2008; Porter and Gawith, 1999; van Ittersum et al., 2003a).

For large scale applications for predicting wheat development, the use of temperature-sums and thermal time requirements is the most practical and successful approach.

As thermal requirements and growth characteristics are cultivar specific, and cultivars vary by geographic regions, regional adaptation of models is the great challenge for wide scale applications. Actual sowing dates and harvest logistics that vary per region generate additional variation in the actual observed wheat phenology in a certain area. Due to necessary simplifications, deviations between model predictions and observation can be considerable (up to 10 days or more) in particular when modelling at large geographic scales (van Bussel et al., 2010). Neglecting all this variability may strongly affect outcomes of wheat phenology models and, consequently, of fungal and mycotoxin models.

Hard (observed) data for thermal time requirements is scarce or not published in a standardized form. However some valuable data can be found in van Bussel et al. (2010), Harrison et al. (2000), McMaster (2005) and Porter and Gawith (1999). However data formats in these publications are not fully standardized. Harrison et al. (2000) also present quite extensive data on flowering dates (not degree-days) for a number of European countries and compare model outcomes with observed data.

On average it takes about 2000 degree-days (DD) from sowing to harvest for winter wheat and 1500 degree-days for spring wheat but there is a large variation in data due to cultivar variation, the way heat sums are calculated and due to variation in farm management factors.

More details for winter wheat are: 100 DD from seed to emergence, 800 DD from emergence to heading, 100 DD from heading to anthesis (flowering) and 600 from anthesis to harvest. More details for spring wheat are: 75 DD from seed to emergence, 700 DD from emergence to heading, 75 DD from heading to anthesis (flowering) and 500 from anthesis to harvest.

Also at the European level, flowering times for wheat are modelled and calculated according to temperature sums that are being calibrated regionally on (mostly) unpublished data (Lavalle et al., 2009). This calibration is necessary as required heat sums vary by region where cultivars are used that respond differently to temperature-photoperiod relations which was also shown in other studies (Yan and Wallace, 1998). Data on thermal heat requirements for wheat in different European regions are available in the Agrophenological database of JRC/MARS, but they can only be used in cooperation with JRC. The models used by the JRC/MARS project use these temperature sums to predict yields and flowering by the WOFOST modelling approach (Lavalle et al., 2009; MARS, on line).

Projections of climate change can be made and phenological shifts can be directly calculated from changes in average temperature regimes. With models as described above (see also Table 5), variation not explained by temperature sums is determined by photosensitivity and genetic make-up of cultivars. When, due to climate change, the choice of cultivars will change in different parts of Europe, predictions however may be systematically biased. It seems likely that other cultivars will be developed and used to optimize production under changing conditions (Yan and Wallace, 1998). Cultivars are promoted by seed companies and chosen by farmers according to regional climatic profiles (seasonal temperature profiles) in order to optimize the length of the growing period and harvest times (Semenov, 2009). The final goals are maximizing production levels, attaining good quality and optimal harvest times. Cultivar diversity at local and regional scale and additional variation in sowing and harvest dates cause considerable variation in flowering and harvest times at different spatial scales. Recently this problem was identified by van Bussel et al. (2010) and may have much impact on the model outcomes of climate projections as variation is often levelled out when modelling at a larger scale and using averages (van Bussel et al., 2010).

1.3.4.4. Day length, vernalisation needs, CO2 and drought stress

Thermal time requirements are often estimated on available experimental data. For modelling purposes , when these data are more general and not collected in the target area for modelling, often the thermal time requirements should be corrected for day-length and vernalisation (chilling needs for winter wheat to initiate spikelet formation) as this may change the phenology of an average variety under region specific conditions (Ewert et al., 1996; Saiyed et al., 2009). In the CERES wheat simulation models this is done by adding a thermal vernalisation requirement and slowing down development at shorter day length (McMaster et al., 2008). Similar corrections are made in the AFRCWHEAT2 model (van Bussel et al., 2010).

With regard to CO_2 levels, several experimental studies have been done on its effect on wheat, but so far no relevant effect on wheat development was found (Slafer and Rawson, 1997).

Because data available for all EU countries are limited, and because the aim of this project is to model the effect of climate change in EU, the approach followed has been to simulate the average performance of wheat according to a criterion applied for all the countries considered.

The main criteria for the selection for the EFSA climate projection has been to choose a general model, applicable all over Europe, validated in several countries with input data simple and readily available from the climate scenarios

The approaches that satisfies this requirements best are the models/data used in the JRC/MARS project (generic model that was validated and made spatially explicit at a European scale) and the AFRCWheat model that is generic and applies simple validated methods to correct for day length and vernalisation on a European scale. Both approaches use simple temperature inputs to simulate the phenological stages necessary to run the disease and mycotoxin models.

For the aims of this project, the JRC/MARS model has been used, which is based on the current cultivar distribution.

Rainfall, radiation and humidity have been assumed to be of secondary importance for the crop modelling, but these factors have been included in the mycotoxin modelling.

1.3.5. Rice growth models

1.3.5.1. Rice Crop Growth Models

Rice cultivation systems are of a high technical level in Europe and, on the contrary of other cereals such as wheat and maize, it is unclear if the quality and safety of rice and derived products could be reduced by mycotoxin contamination. Few data are available on the occurrence of mycotoxigenic fungi on the rice plants and heads during the growing season and on the harvested grain during storage and even during processing. However, fungi may infect the rice in all growth stages, from sowing up to harvest, and some rice growth stages and parts of the plant could be more vulnerable to infection than others. Both crop and fungal disease development and growth depend on environmental conditions, such as weather. Hence they are likely to be influenced by climate changes.

Understanding the development and growth of both rice plants and pathogenic fungi during the cultivation period, the factors involved, as well as their interaction, is important to predict and manage risks for mycotoxin contamination. Crop growth models are an essential part to predict the potential risk conditions for fungal infection and toxin formation.

European rice is usually grown under flooded conditions ("paddy" rice), also to achieve thermal regulation. The floodwater strongly characterizes the biophysical structure of paddy rice fields, influencing the vertical thermal profile, the nutrients use efficiency and leaching, the emission of greenhouse gasses, the presence of weeds and crop diseases (Confalonieri et al., 2005; Confalonieri et al., 2009b; Huguenin-Elie et al., 2003). Especially in temperate rice areas (northern Italy, northern Japan, northern Korea, northern China and southern Australia), the floodwater effect on vertical thermal profile plays a major role in preventing cold shocks during the emergence and pre-flowering stages.

As mentioned above, rice crops are commonly grown in Europe as a lowland direct-seeded crop under fully irrigated conditions and the growing cycle is mainly determined by temperature constraints and cultivars characteristics (Casanova et al., 1998). The ecological conditions of rice cultivation in Europe can vary greatly among the most important growing areas. In northern Italy, the climate of rice production is temperate-continental, with a cold winter and warm summer and main rainfall occurring during the first stages of the crop growth (April-June) and the harvesting period (September-October) (FAO, 1996). In most of the other European countries the climate is sub-tropical (Mediterranean climate) with a dry summer and warm, dry, clear days and long growing season. However, in general,

the climate is characterised by long days, high solar radiation and relatively large diurnal temperature fluctuations (Hill et al., 1991). When directly-seeded, the same rice cultivars have lower tiller and require greater nitrogen inputs to produce the same yield (Dingkuhn et al., 1991).

On the other hand, the rice productivity in European regions can be affected by numerous other constraints. Most of them are related to water availability, and biotic and environmental stresses. Crop growth models normally aim at predicting yield. Therefore, in rice, it is important for models to be parameterised and validated in different agro-environments (START, 1997). Moreover, for rice crop, a key point of model studies is to investigate the relationship between crop management, productivity and environmental impact of paddy rice systems (McMennamy and O'Toole, 1983).

However, the climate remains the most limiting factor considering that the most developed European rice area is located in northern Italy (> 45°N) where cold temperatures (5 °C or less) can occur at sowing (in April) causing damage to seedlings and poor establishment in rice fields. Sudden decrease of temperature or strong diurnal variations can occur at flowering, during thunderstorms in August, causing spikelet sterility and/or more favourable conditions for fungal disease attacks. Thunderstorms and wind during the ripening stage may cause severe lodging of the tallest varieties. Therefore, also in rice, phenology has always to be included in yield predicting models (Confalonieri et al., 2009b), which are often also used to predicting the growth stages that are sensitive to fungal infection in rice.

1.3.5.2. Rice Phenology

The growth of rice plant is divided into three phases:

(i) **vegetative** (germination to panicle initiation);

(ii) **reproductive** (panicle initiation to flowering);

(iii) **ripening** (flowering to mature grain).

The differences in growth duration depend on variety. Vegetative phase length can vary from 60-65 days, reproductive phase from 30-35 days and ripening length can vary from 15-40 days.

These 3 phases consist of a series of 10 distinct growth stages (GS) shown in Figure 8, numbered and described as follows:

GS 0 is from germination to emergence; GS 1 is called seedling; GS 2, tillering; GS 3 is stem elongation. These first 4 stages make up the **vegetative phase**, the first phase of rice plant growth.

The **reproductive phase**, the second phase of rice growth**,** starts with GS 4, panicle initiation to booting; GS 5follows, which is heading or panicle emission and GS 6 is flowering, the last step.

Finally, GS 7 through 9 correspond to the **ripening phase**, the last phase in the development of the rice plant, and, in particular, GS 7 is the milk grain stage; GS 8, the dough grain stage, GS 9, the mature grain stage.

Temperature is the main driving variable for phenological development (Van Keulen et al., 1982). Critically low and/or high temperatures define the environment where the life cycle of the rice plant can be completed. Critical temperature thresholds are:

(i) low temperatures around 15 °C from the seedling stage to panicle initiation

(ii) low temperatures around 20 °C or high temperatures around 35 °C at flowering which could induce sterility during pollination (about 80 days after planting).

Within the critical low and high temperature range, temperature influences both the rate of development of leaves and panicles and the rate of grain filling, thereby affecting the growing season length of a rice variety, eventually determining the suitability of that variety to the environment (Bachelet and Gay, 1993). Increased temperature speeds up plant development, but decreases the length of the grain filling period. According to Bachelet and Gay (1993), for a 120-day rice variety, the average daily temperature during the 55 day-long vegetative period should be around 22 °C and the temperature during the reproductive period (about 21 days) should be around 24 °C. Harvest occurs after an approximate 35-day period of grain filling and maturation with an average daily temperature of 24 °C.

In photoperiod-sensitive varieties, day-length determines the induction of flowering as well.

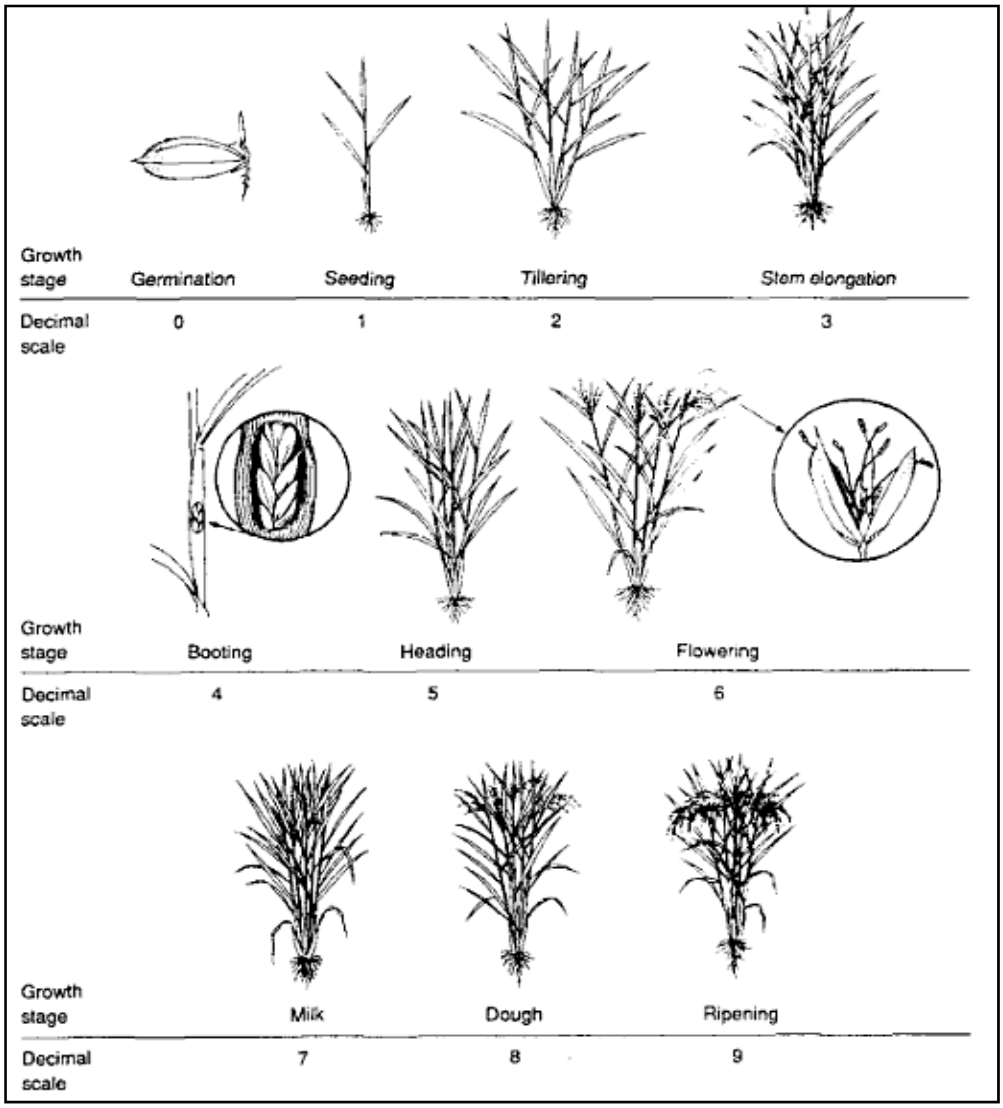

Figure 8: Growth stages of rice, adapted from Reissig et al., (1986), on a decimal scale (Zadoks et al., 1974)

1.3.5.3. Rice models

Crop growth models in general consider all the factors that affect the physiology and development of the plants (Brisson et al., 2003).

As a general classification, three major groups of process-based models can be distinguished, depending on the approach used for daily accumulation of biomass under non-limiting conditions, temperature and radiation being the only factors limiting growth.

The first group includes the family of models based on net carbon assimilation as a balance of gross CO_2 assimilation and maintenance and growth respiration; SUCROS (Van Keulen et al., 1982), which has been the basis for derived models is an example (i.e. WOFOST, by Van Keulen and Wolf, (1986)). This approach is conceptually sound and comprehensive enough to draw attention to gaps in understanding and for analyzing processes at various plant components (Confalonieri and Bechini, 2004). Its usage implies considerable efforts because of the high requirements in terms of parameters and input variables. Moreover, it has not proved to be more accurate than simpler approaches when biomass estimates were evaluated against actual data (Jamieson et al., 1998).

Other approaches to daily biomass accumulation are based on the concept of net photosynthesis: crop biomass is considered proportional to one (or both) of two main driving factors involved in the photosynthetic carbon fixation, i.e. intercepted radiation and transpired water. Models based on intercepted radiation are the second major group of models. In this case, aboveground biomass (*AGB*) accumulation under optimal growth conditions is linearly related to cumulative light interception (Monteith, 1977). The core of this approach is the concept of radiation use efficiency (*RUE*), a parameter used to convert intercepted solar radiation into *AGB*. Part of the APSIM crop models (Keating et al., 2003), and the models of the CERES family (Jones et al., 1984) basically implement a *RUE* based approach.

The third major group of models include crop growth equations based on the transpiration use efficiency (*TUE*) (Tanner and Sinclair, 1989). The *AGB* is computed daily by multiplying a biomass/transpiration coefficient (theoretically species- or variety-specific) by the ratio of potentially transpired water to the mean vapour pressure deficit (*VPD*). A problem of this approach is associated to the division by *VPD*, which may result into infinite values of estimated crop growth for nearly to zero *VPD* values. This is why the best known model adopting this approach, Crop-Syst (Stockle et al., 2003), also computes each day a second *AGB* value using the *RUE*-based approach and takes the minimum of the two.

ORYZA1 model. For rice, a model frequently used has been ORYZA1 (Kropff et al., 1994), an explanatory model to simulate rice growth, development and leaf area index (LAI) under potential production. ORYZA1 was firstly developed based on data from the Philippines where conditions differ widely from those prevailing in European rice areas. However, later, Casanova et al. (2000) adapted the model to the Mediterranean conditions.

Effective temperature for phenological development. It has been observed in many crops that the rate of development is linearly related to the daily mean temperature above a base temperature up to an optimum temperature, beyond which the rate decreases, again linearly, until a maximum temperature is reached. For temperatures below the base temperature or above the maximum temperature, the rate of development is zero. Three "cardinal" temperatures can therefore be identified: base temperature (T_{base}; °C), optimum temperature (T_{opt}; °C), and maximum temperature (T_{high}; °C). For rice, these values are typically 8, 30, and 42 °C, respectively (Gao et al., 1992). This "bilinear" response is generally observed only when the daily temperatures are constant (i.e. in a controlled environment); if the temperature fluctuates between a minimum and a maximum value, as is the case in field environments, the response becomes more curvilinear, particularly near each cardinal temperature.

Although this curvilinear response to daily mean temperature can be described by complex exponential equations i.e. (Gao et al., 1992; Yin, 1996), the simpler approach used by Matthews and Hunt (1994) in their cassava model was used in ORYZA1. In this approach, they assume that the response of development rate to temperature over short time periods, such as one hour, is described by the bilinear model, and that the response to daily mean temperature is achieved by superimposing onto this model a temperature response approximated by a sine function alternating between the daily minimum (T_{min}; °C) and maximum (T_{max}; °C) temperatures.

Regarding low temperature and crop survival, usually when in more than 3 days the average temperature is lower than 12 °C the crop dies.

Phenological development rate. The development rate of the crop is calculated based on development rate constants for the different growth stages, the daily increment in heat units (HU; °Cd/d), and the photoperiod.

The life cycle of the rice crop is divided into four main phenological phases:

(i) The basic vegetative phase (BVP), from emergence to the start of the photoperiod-sensitive phase.

(ii) Photoperiod-sensitive phase (PSP), from the end of the basic vegetative phase to panicle initiation.

(iii) Panicle formation phase (PFP), from panicle initiation to (50 %) flowering.

(iiii) Grain-filling phase (GFP), from (50 %) flowering to physiological maturity.

The photoperiod is calculated from the day-length +0.9 to account for the effect of low radiation levels after sunset and before sunrise. Each of these four phases has a variety-specific development rate constant, which is the inverse of the temperature sum required to complete a specific phase at the optimum photoperiod. Differences between varieties in total crop duration are usually caused by differences in the duration of the BVP rather than the other phases. Suboptimal photoperiods (day-length; DL; h) less than the optimum photoperiod (MOPP; h) will result in a longer photoperiod-sensitive phase, which depends on the variety.

Drought stress and development rate. Drought in the vegetative stage of development delays flowering. Wopereis et al. (1996) found that the delay in flowering decreased when drought occurred at later growth stages. In their experiments, postponement of flowering was in reasonable agreement with the number of days between the date of zero leaf expansion and the recovery from drought. This indicates that, if the soil is too dry to produce new leaves, the development rate of the crop is brought to a standstill as well. Therefore, the leaf expansion reduction factor is used to simulate the effect on delayed flowering in ORYZA1 and varies with changes in water balance to simulate water-limited conditions.

The adaption of ORYZA1 by Casanova (2000) to the Mediterranean conditions was performed by calibrating and testing the performance of the model for direct-seeded fully irrigated rice. ORYZA1 was calibrated and validated with field data of two cultivars, a short-grain (Tebre) and a long-grain cultivar (L-202), grown in various years in the Ebro Delta of Spain. Phenological development of the rice crop, daily dry matter production and leaf area development were calibrated. Tebre and L-202 had no significant differences in the total length of the development period. The temperature sum from seeding to maturity, with a temperature base of 10 °C was on average 1650 degree-days for both cultivars. The pre-heading period, however, was longer and the post-heading period shorter in L-202 than in Tebre. This induced differences in translocation characteristics, spikelet number per unit area,

weight of the grains and harvest index. The model simulated rice growth very accurately until flowering. After flowering, however, divergences appeared and increased especially at the yellow ripe stage. This reduction of growth rate was usually accompanied by an increase in the relative death rate of leaves and the drying of the grains. The main source of error was considered to be due to a limited understanding of the ripening and sink limitation processes. A considerable gap between potential and observed yield has been noticed.

WARM model. WARM (Water Accounting Rice Model) used by Confalonieri et al. (2009b) was a novel model for paddy rice simulations, compared to the rice models previously available i.e. CERES-Rice (Singh and Kumar, 1993), ORYZA1 (Kropff et al., 1994), maNageRice (Angus et al., 1996), CRISP (Anastacio et al., 1999), where distinct processes of rice physiology were taken into account. Although some authors, i.e. Dingkuhn et al. (1995) and Nishiyama (1995), have underlined the importance of the floodwater effect on temperature (the latter being one of the most important driving variables in cropping systems models), none of the abovementioned rice models includes routines to simulate the impact of floodwater on the thermal profile affecting the crop.

On the contrary, WARM includes modules for the simulation of processes not accounted for in other rice models, such as the micrometeorological module TRIS (Temperature for Rice Simulations) for the simulation of floodwater effect on vertical thermal profile proposed by Confalonieri et al. (2005); a module for the simulation of rice blast diseases, responsible for serious leaf damage and consequently yield losses; a module for the simulation of spikelet sterility due to cold shocks during the pre-flowering period (Confalonieri et al., 2009b).

The presence of these specific modules makes WARM particularly suitable for the simulation of rice in temperate areas, like those characterizing the European rice districts. Moreover, the data requirements in terms of input variables and morpho-physiological parameters describing the different rice varieties are lower in comparison to most of the other approaches, making the model effective in case of large scale simulations. The model has been successfully evaluated against real data in different environments, i.e. China and Italy (Confalonieri et al., 2009a), also in comparative studies with other models of large use worldwide (Confalonieri et al., 2009a), and is currently being used by EC for rice yield forecast at European level. Owing to the explicit intention of reproducing the features of European rice systems and the novelty of some of the modelling approaches developed in WARM, Confalonieri et al. (2010b) tried to better understand model behaviour in Europe, focusing on the sensitivity of the biomass output to the model inputs. The analysis was carried out using three years of meteorological data, with average, highest and lowest continentality, for each of the main European rice districts. The output considered was aboveground biomass at maturity, simulated at five rice districts of different countries (France, Greece, Italy, Portugal, and Spain) for years characterized by low, intermediate, and high continentality. Two sets of parameters were considered:

a) the parameters involved with net photosynthesis, namely, Radiation use efficiency (**RUE**); Extinction coefficient for solar radiation (**k**); Base temperature for growth ($\mathbf{T_{base}}$); Optimum temperature for growth ($\mathbf{T_{opt}}$); Ceiling temperature for growth ($\mathbf{T_{max}}$);

b) the parameters involved with aboveground biomass partitioning and leaf area index, namely, LAI_{ini} Initial leaf area index; LA_{ini} Initial specific leaf area; LAT_{ill} Specific leaf area at tillering; Rip_{L0} Partition coefficient to leaf at early stages; LeafLife Leaf duration; H_{max} Maximum panicle height.

The data obtained showed that Radiation use efficiency (RUE), optimum temperature (T_{opt}), and leaf area index at emergence (LAI_{ini}) ranked in most of the combinations site x year as first, second and third most relevant parameters, respectively (Confalonieri et al., 2010b).

Other models. A number of further rice growth and productivity models were recently developed by several groups. A description of some selected models follows.

Maruyama and Kuwagata (2010) coupled a crop growth model with a simple land surface model to estimate the effect of changes in the growing season for rice on the energy balance and water use of rice paddy fields. The crop growth model consisted of calculations of phenological development (Ps), growth of leaf area index (LAI) and canopy height (h). The land surface model consisted of calculations of energy balance, radiation transport and stomatal movements using the output of the crop growth model (Ps, LAI, h). Using a coupled model, the energy fluxes and water/canopy temperatures of rice paddies were calculated from climatic data only. Using the proposed model, the water use by rice paddies can be estimated for given climatic conditions and growing seasons, which makes it possible to modify the growing season and water management for climate change.

RiceGrow. With the objective to define a rice growth simulation model that could provide a systematic and quantitative tool for predicting growth, development and productivity of rice under changing environmental conditions, Tanga et al. (2009) developed a model called RiceGrow taking in account that growth and yield formation in rice depend on integrated impacts of genotype, environment and management. They tried to overcome the difficulties of the existing rice models which perform well, but need a large number of parameters to be estimated. From the experience in modelling wheat, they used the physiological development time (PDT) as a scaler for phenology and a partitioning index for organ growth in order to have fewer parameters that provided good predictability and applicability. The model included seven sub-models for simulating phenology, morphology and organ formation, photosynthesis and biomass production, dry matter partitioning, yield and quality formation, water relations and nutrient balance. The RiceGrow model was compared with the existing ORYZA2000 model, showing that both provided satisfactory estimates for phenology, shoot biomass and yield. Overall, RiceGrow can be used to predict rice growth and development with varied genotypes, environmental conditions and management practices for multiple uses including scientific understanding, policy formulation and optimizing crop management.

Impact of CO_2. An interesting evaluation of the impact of elevated CO_2 and temperature on rice yield in eastern India was simulated by using the ORYZA1 and the INFOCROP rice models by Krishnan (2007). The crop and weather data which were used originated from 10 different sites which differed significantly in their geographical and climatological factors. For every 1 °C increase in temperature, ORYZA1 and INFOCROP rice models predicted average yield changes of -7.20 and -6.66 %, respectively, at the current level of CO_2 (380 ppm), but increases in the CO_2 concentration up to 700 ppm led to the average yield increases of about 30.73 % by ORYZA1 and 56.37 % by INFOCROP rice. When temperature was increased by about + 4 °C above the ambient level, the differences in the responses by the two models became remarkably small.

For the fungal infections, the phenological sequence of growth stages during the season is important. Although advanced crop growth models, such as those abovementioned, often include phenological aspects and all rice growth factors, they are often too complex to be linked to predictive models for fungal infection and mycotoxin production. Simple models that focus on phenology are more relevant in this context. Such models are often primarily driven by temperature. However, the impact of other factors, especially water availability impact, should be known as well, to ensure the models give accurate predictions, and can be used over large areas, with different weather conditions and under future climate scenarios. A model to be selected for studies at European level must simulate also the impact of floodwater on the thermal profile affecting the rice crop, since in Europe rice is usually grown under flooded conditions. Therefore, for rice simulations, the use of WARM model has been adopted in this contest (Confalonieri et al., 2005).

1.3.5.4. Climate change studies and rice cultivation shift area

For the effects that the climate can have on rice cultivation shift area, literature have been screened with the terms "rice" and "climate", "distribution" and "growing area". Some publications are available which show projected future rice distributions but in particular production levels (which probably predict economic profitability to grow rice) such as those of Bachelet and Gay (1993), Matthews and Wassamann (2003), Shimono (2008), and Shimono (2010).

All these studies showed a positive impact of climate change on yield with respect to the increasing levels of atmospheric CO_2. With respect to temperature, global warming seems to allow a northward expansion of rice growing areas and a lengthening of rice growing seasons which are now constrained by low temperatures. In some areas, it may even be possible to grow two annual rice crops instead of only one. However, the acceleration of the development process can also result in incomplete grain filling and reductions in yield. Shimono (2010) found that increased temperature reduced maturation time and induced a linear decrease in yield while biomass remained mostly unaffected. Rice disease incidence may also be severely affected by modifying the frequency of occurrence of the optimal infection temperature. Finally, the rhizosphere flora is sensitive to high temperatures. Because free-living nitrogen fixers are abundant in the rice root zone, a change in temperature could affect the paddy nitrogen cycle. By evaluating the suitability of rice models for assessing the impact of global climate change on rice production, Shimono et al. (2007) used four physiologically-based rice models (RICEMOD, CERES-Rice, MACROS, RICESYS) and showed their potential to predict rice responses to increased temperature. The results clearly indicate that all four models predicted a decrease in rice yield due to a temperature increase (from 25 °C to 30 °C, the yield estimated reduction ranged from 12 to 62 %). However, as MACROS and CERES-Rice simulated more realistic responses to temperature than the others, and because RICEMOD and RICESYS did not simulate the effect of CO_2 level, the study concluded that the first two were the most suitable for climate change studies.

1.4. Host pathogen interaction

The pathosystem cereals-*A.* section *Flavi* is complex and hardly unknown, at least regarding fungal growth and AFs production in wheat and rice. Due to similarities between maize and other cereals regarding the infection of other mycotoxin producing fungi, mainly those belonging to *Fusarium* spp., it seems reasonable to suppose a similar behaviour of fungi on different crops. Flowering could be assumed as the crucial growth stage for fungal infection and damages caused to ears by pest insects as enhancing factors for aspergilla dissemination, new infections occurrence and AFs production.

The description of factors influencing *A.* section *flavi* growth and AFs production was approached following the infection cycle of fungi. This is suggested because modelling needs to follow the system analysis, where the fungi-host system is considered; it means that the steps described in this paragraph have been the steps considered in modelling. Therefore, all factors relevant for fungal growth and AFs production supported by quantitative data were included in the model.

1.4.1. Steps in the infection cycle

1.4.1.1. Source of inoculum

Aspergillus flavus and *A. parasiticus* can be considered plant pathogens but, from an ecological point of view, living tissues represent only a minor substrate for these soil borne filamentous fungi. *Aspergillus flavus* and *A. parasiticus* are saprophytic during most of their life cycle and grow on a wide variety of substrates including decaying plant and animal debris. Communities of section *Flavi* differ by region in both species composition and AFs producing potential. In particular, *A. flavus* species can be divided in two sub-species on the bases of sclerotia size: S strains are those with small sclerotia (< 400 μm) and able to produce high amounts of AFs while L strains produce fewer and larger sclerotia (> 400 μm) and variable levels of AFs (Cotty, 1989). S strains incidence has been shown as positively correlated with clay content and negatively correlated with sand content of soils (Jaime-Garcia and Cotty, 2006).

These fungi can overwinter in soil as mycelia or conidia (Angle et al., 1989). *Aspergillus flavus* can also produce sclerotia able to germinate on the soil surface when weather conditions become appropriate (Wilson et al., 1989).

Sclerotia and conidia of *A. flavus* and *A. parasiticus* have a high survival rate; when buried at a depth of 10-12 cm for a period up to 36 months in sandy field soils in two different areas (Illinois and Georgia, USA), their survival remained high. In particular, substantial losses of conidial inoculum were recorded after the first year of burial in Georgia and after the second year of burial in Illinois. In general, conidia of *A. parasiticus* had a lower survival rate in soil than conidia of *A. flavus* (84 *versus* 93 % respectively). Regarding *A. flavus* sclerotia, the number of propagules generated in soil varied according to strain and location, but the number was maximum after the first growing season (April to October). Over the 3 years considered in the over cited study, the rates for sclerotium survival in Illinois were similar to the overall rates in Georgia (88 % *versus* 89 %), however none of the sclerotia recovered after 36 months germinated sporogenically on moist sand (Wicklow et al., 1993). Soil populations of *A. flavus* under maize cultivation can range from 300 to 300,000 colony forming units (CFU)/g soil.

Aspergillus flavus and *A. parasiticus* can grow at temperatures from 12 °C to 48 °C and at water potentials as low as – 35 MPa (0.77 a_w) (Klich et al., 1994) even if optimal temperatures for growth are from 25 °C to 42 °C; for this reason, these fungi can be considered semithermophilic and semixerophytic (Payne, 1998).

In particular, a study conducted during the wet and dry seasons in Philippines, using different concentration of *A. flavus* inoculum in soil, showed that the highest infection and contamination of maize were observed with the highest level of inoculum indicating a direct relationship between soil population and extent of infection and AFs contamination occurring in pre-harvest maize. Moreover, the extent of infection and AFs contamination were generally lower during the wet than the dry season trials (Garcia et al., 1996).

Under conditions of high temperature and low a_w, conditions associated with drought in temperate agricultural crops, *A. flavus* and *A. parasiticus* become very competitive and may become the dominant fungal species in the soil (Payne, 1998).

The populations of these organisms on plant and in the soil are also dependent upon how well they can compete with the other microflora present (Payne, 1998). However, comparing maize cobs left in field and soil as sources of *A. flavus* inoculum, it has been seen the higher importance of the first respect to the latter. Effectively, in a study conducted in Texas, maize cobs were the major sources of *A. flavus* inoculum: maize cobs from the previous season contained over 190 times more *A. flavus* propagules than the soil from the same field and also in older maize cobs (2 years old) still retained 45 times more propagules than the soil. The quantity of *A. flavus* in maize cobs decreased with maize cob age, but in some areas, where crop rotations are common and minimal tillage is applied, they can represent a long-term source of inoculum (Jaime-Garcia and Cotty, 2004).

1.4.1.2. Inoculum dispersal

Aflatoxigenic fungi residue in soil as conidia, sclerotia and hyphae, which act as primary inocula for infecting maize through wind and insect dispersal (Horn, 2003). The teleomorph (sexual stage) of *A. flavus* is not known and conidia are assumed to be the primary inoculum. From soil, the airborne conidia are deposited on the silks and kernels (Payne, 1998); no spore dispersal was observed in rainy days in Italy (Battilani et al., unpublished data). Infected crops periodically replenish soil populations during drought years (Horn, 2003).

Some studies revealed that airborne conidia of *A. flavus* are present during all the year in Sudan at different concentrations (highest from May to August) (Abdalla, 1988) and they can run short distances, from 2 to 14 m decreasing linearly the concentration along the way, independently from the growing season and place considered (Olanya et al., 1997). This linear decrease was also associated with linear declines in the incidence of *A. flavus* infection on leaves, silks and kernels of maize plants with increasing distance from each place considered as source of inoculum (Olanya et al., 1997).

During a survey organized in USA in 1984, airborne conidia were detected throughout the period from maize silking to harvest maturity, with the greatest number of conidia detected at all locations considered during the first sampling period (mid-August). This coincided with the period of onset of maize silking, which is the optimum time for plant infection by *A. flavus* (Marsh and Payne, 1984b).

In a study conducted in India, in two different locations, it has been demonstrated that the percentage incidence of *A. flavus* in the aerosphere and in the soil of maize fields was significantly affected by season x location interaction. Moreover, also the ratio of non-toxigenic and toxigenic strains of *A. flavus* varied with source and location of isolations (Bilgrami and Choudhary, 1993).

In particular, considering aerosphere of maize fields in India, the percentage of *A. flavus* was highest (63 %) during monsoon season (July-September) followed by summers (45 %) and lowest (24 %) in winter (January-March) independently from place considered. Regarding soil samples, the frequency of isolation of *A. flavus* was also high during the monsoons (76 % and 69 % in two locations) and the lowest was observed during the summers (Bilgrami and Choudhary, 1993). The two overriding conditions that influence AFs contamination are temperature and moisture. High temperature and drought conditions increase the airborne inoculum of the fungus. The increased growth of the fungus at higher temperatures is presumably related to its relatively high optimum growth temperature. The fungus can grow over a wide range of temperatures (from 12-48 °C) with an optimum at 37 °C. The higher temperatures and drought conditions also may favour *A. flavus* over other fungi because of its ability to grow on substrates with a low water activity (Anonymous, 2003; Payne, 1998). Although no significant relationship between airborne populations of *Aspergillus* and air temperature was established (Li and Kendrick, 1995), *Aspergillus* spp. appear most abundant between latitudes 26° to 35° north or south of the equator. Thus, the fungus is more common in subtropical and warm temperature climates (Anonymous, 2003). Statistical analysis showed that *Aspergillus* is negatively correlated with relative humidity and positively correlated to wind speed (Li and Kendrick, 1995).

An array of arthropod species also contributes to the dispersal of these aspergilli as they attack and feed on the developing grains (Scully et al., 2009).

Insects may contribute to the infection of kernels in four ways:

(i) transport primary inoculum to the ears: insects may be conveyors of fungal spores, but the ear may already be infected with *A. flavus* as a result of high spore loads during the receptive period of silking;

(ii) move inoculum from the silks into the ear;

(iii) disseminate inoculum within the ear;

(iv) facilitate colonization and the infection by injuring kernels: insects are able to facilitate the infection process by wounding intact tissue and providing more infection sites.

As demonstrated by Wildstrom (1979), wounding may also allow kernels to dry down to moisture levels that support *A. flavus* growth and subsequent AFs production.

However conidia of *A. flavus* have been shown to be airborne so the relative contribution of insects to the initial colonization of the ears is not clear. Probably, under favourable environmental conditions, insects are not required to infect the ear, distribute it within the ear, or provide a site for the entry of the fungus. In contrast, when the environmental conditions are less favourable for *A. flavus*, only a few kernels may be colonized in the absence of insect injury (Payne, 1998).

Pollen seems to play a critical role in the establishment of *A. flavus* on external silks. Maize pollen is a source rich of carbohydrates, aminoacids and minerals (Linskens and Pfahler, 1973; Pfahler and Linskens, 1971, 1974) and acts as an excellent substrate for *A. flavus* and *A. parasiticus*. Conidiophores were abundantly and rapidly produced (in less than 24 hours) by inoculating *A. flavus* onto moistened pollen grains held at 34 °C in the laboratory (Marsh and Payne, 1984a).

1.4.1.3. Spore germination

In a greenhouse experiment, where ears of a single maize hybrid were artificially inoculated with *A. flavus* at different growth stages, conidia on the yellow-brown silks germinated in 4-8 hours. Germination occurred first nearest the pollen grains, and the hyphae spread rapidly across the silk, producing extensive growth and lateral branching. Differently, on green not pollinated silks only a few conidia germinated 24 hours after inoculation and they failed to establish significant mycelial growth. Fungal growth on brown silks was comparatively scarce and often localized on pollen grains (Marsh and Payne, 1984a).

Days necessary for spore germination vary depending on how the ecological conditions fit with fungal needs. In particular, depending on the strain of *A. flavus*, the number of days necessary to have spore germination varies from 8 to 16 at 0.78 a_w and optimal temperature (33 °C), increasing to 95 days when temperature arises or decreases of 7 °C. At optimal conditions, the spore germination was obtained after 1 day (Ayerst, 1969).

At optimal water activity conditions (> 0.95 a_w) and temperature in the range of 15-45 °C, spore germination is always possible. Decreasing a_w level, the percentage of germinated spores decreased and definitely ceased at 0.80 a_w independently from temperature (Marín et al., 1998; Schmidt-Heydt et al., 2010).

A more recent *in vitro* study, with a large range of a_w (from 0.75 to 0.996) and 3 temperature tested (25 °C, 30 °C and 37 °C), established that germination of *A. flavus* and *A. parasiticus* is more rapid at the highest level of a_w used (0.996 and 0.99) at 37 °C. Little difference exists in germination times at 25 °C and 30 °C, except at low a_w where at 30 °C was faster (Pitt and Miscamble, 1995).

The minimum a_w observed for *A. flavus* and *A. parasiticus* spore germination was identical at each temperature and was 0.814, 0.810 and 0.816 a_w at 25 °C, 30 °C and 37 °C respectively on artificial media (Pitt and Miscamble, 1995).

1.4.1.4. Infection

Insects and growth stage - In the past there was the conviction that the pre-harvest infection of maize by *A. flavus* could happen only after ears had been predisposed by insect injury since very often visible sporulation were located in coincidence with areas of insect damage (Butler, 1947). Further studies provided evidence for a role of insects in the infection process, showing positive associations between insect damage and infection and sporulation by *A. flavus* and AFs levels (Wilson et al., 1981). The incidence of *A. flavus* and *A. parasiticus*, as well as the level of AFs, result comparatively higher in insect-damaged maize samples than undamaged samples (Sinha and Sinha, 1992). It is well known that poor pest control increases insect damage, facilitating fungal infection and AFs contamination (Plasencia, 2004). However, data from several investigations failed to show any relationship between insect damage and AFs or the incidence of *A. flavus* (McMillian et al., 1978; Wilson et al., 1981) since

kernel and ear samples essentially free of insect damage, but with high amounts of AFs have been found (Lee et al., 1980; Lillehoj et al., 1980).

Several studies determined that the colonization of silks and kernel surfaces occurs soon after silk emergence and may continue and increase throughout the season. The fungus can be brought to the kernels surfaces by insects or can colonise silk tissues and grow down into the ear (Jones et al., 1980; Marsh and Payne, 1984b; Payne et al., 1988). Although much of the hyphal growth appears on the surface of the silks, *A. flavus* can penetrate through the silks directly or through cracks and intercellular gaps (Payne, 1998), but usually even when colonization of kernel surfaces by *A. flavus* is extensive, internal infection is low (Marsh and Payne, 1984b), in some cases below 1 % (Windham and Williams, 2007).

In a study conducted on maize in North Carolina (USA), in field and greenhouse trials, *A. flavus* has been isolated from silks throughout the season. Three sampling dates were considered: milk stage (31st July), dough stage (9th august) and dent stage (14th august). At milk stage, *A. flavus* was isolated from the silks of 30 % of the ears harvested and among these ears with colonised silks, 100 % had *A. flavus* in silks from the tip section, 67 % from the middle and 33 % from the base. At dough stage, the percentage of ears with silks colonized by *A. flavus* approximately doubled to 72 % and fungal growth on kernels was detected in 47 % of the ears. At early dent stage, about the same percentage of ears (75 %) had silks colonised by *A. flavus* and 50 % revealed colonization of kernels by *A. flavus*. The presence of insect damage (European Corn Borer - ECB and corn ear worm) over the three sampling dates progressed from 20 % of the tip, 10 % middle, and 10 % base at milk stage; 100 % tip, 25 % middle and 35 % base at dough stage; to 100 % tip, 85 % middle and 60 % base at dent stage (Marsh and Payne, 1984b).

The fungus colonises the silks first, then the glumes (by the milk stage), the kernel surfaces and, rarely, the cob pith (Marsh and Payne, 1984a). A recent study seems to demonstrate that *A. flavus* and *A. parasiticus* are able to infect ears trough systemic movements of these two fungi in stalks (Windham and Williams, 2007).

Although damage is not a prerequisite for AFs formation, the incidence of *A. flavus* and levels of AFs contamination were higher in damaged kernels (Diener et al., 1987). Kernel damages can come primarily from insects, but also from mechanical damages from farm equipment, birds and from a variety of environmental factors (Bradburn et al., 1993).

Stage of growth and location of kernels on maize ears can be other important factors in the process of kernel infection. The results of a study managed in Egypt showed positive correlation between maize growth stage and kernel infection; the artificial inoculation of maize with these fungi showed to reduce seeds germination, protein and total nitrogen content (Amer, 2005).

Stress factors - Stress on developing maize facilitates infection by the fungi, production of mycotoxins and grain contamination. Drought, excessive heat, inadequate plant nutrition, insect feeding on developing kernels, weeds presence, excessive plant populations and other plant diseases can produce plant stress and facilitate the infection of maize grain by mycotoxin-producing fungi (Bruns, 2003).

In particular, field studies demonstrate that the reduction of drought stress by irrigation reduces AFs contamination in maize and that drought-tolerant maize cultivars result in significantly less AFs contamination in the field under drought conditions (Chen et al., 2004).

Competition - Another important factor is the ability of *A. flavus* and *A. parasiticus* to compete with other fungal species possibly present on maize kernels; species interactions may be important in determining if and to what extent AFs are produced in kernels (Weckbach and Marth, 1977). The

status of individual fungal colonists as interference competitors and the sequence in which these colonists become established within the kernel may contribute to the considerable variation in AFs contamination among field samples (Wicklow et al., 1980).

1.4.1.5. Fungal growth

Several factors may influence the development of *A. flavus* and *A. parasiticus* on maize, first of all meteorological conditions registered in the area of maize cultivation; this is because both a_w and temperature can influence significantly fungi activity.

Various papers report that both high temperature and low humidity stimulate the growth and AFs production of aspergilli (Abbas et al., 2004; Payne, 1998; Scheidegger and Payne, 2005). Under conditions of drought stress and high temperatures, *Aspergillus* outcompetes other microflora on seeds of maize, peanut, cotton and nuts. Not only do these conditions favour the aflatoxin-producing fungi, but they also favour insect activity and often compromise the defence system of the host plant (Payne, 1998).

In 1969, Ayerst described well the dynamics of different fungi, including *A. flavus*, with an experiment *in vitro* on artificial medium. *Aspergillus flavus* resulted to have optimal temperature for growth at 33 °C and a_w higher than 0.98, with 12 °C and 43 °C being limit temperatures and 0.78 the limit for a_w.

Regarding growth rates, wide differences have been reported, depending on ecological conditions going from 0.1 mm/day at 0.78 a_w, and 15-42 °C to 10 mm/day at 0.98 a_w and 33 °C (Ayerst, 1969).

Similar results were obtained by other authors (Holmquist et al., 1983) that considered also *A. parasiticus* in *in vitro* trials and confirmed 33 °C as the optimal temperature for growth of these fungi. In particular, they found that a_w has a higher effect on fungal growth respect to temperature and that growth for both fungi considered was highest at 0.99 a_w. As the a_w of the medium decreased, growth rate decreased. However, the minimum a_w for growth and the level of growth were temperature dependent. Both fungi considered responded similarly to all combinations of a_w (from 0.85 to 0.99 a_w) and temperatures (from 15 °C to 33 °C), but *A. flavus* grew at a slightly slower rate than *A. parasiticus*.

At temperatures higher than 22 °C, growth occurred at all a_w levels tested and decreased linearly as a_w decreased from 0.99 to 0.85. At 15 °C, growth was observed only from 0.95 a_w but not at 0.90 a_w and at 0.80 a_w (Holmquist et al., 1983).

In the *in vitro* study of Pitt and Miscamble (1995), the optimum a_w for growth resulted in the range from 0.96 to 0.98 at 25 °C, 0.985 at 30 °C and 0.96 at 37 °C. Growth rates at 25 °C for *A. flavus* and *A. parasiticus* were very similar over the entire range of a_w considered, except that *A. parasiticus* grew rather more rapidly at high a_w than *A. flavus*. The minimum a_w requested for growth was higher than that for germination (0.83 *versus* 0.81) at all the considered temperatures.

The possible presence of other fungi on ears able to compete with *A. flavus* and *A. parasiticus*, is another aspect to be taken into consideration studying their growth. In particular, *F. verticillioides* appears to compete with *A. flavus* on the maize ear; they can be dominant in years with temperate weather and with high temperature and drought stress respectively. *F. verticillioides* could interfere with infection and AFs accumulation in developing maize seeds (Wicklow et al., 1988).

A negative correlation between the presence of *A. flavus* and *F. verticillioides* has been shown by Hill et al. (1985).

Considering fungi of the same genera present on maize, *A. flavus* was dominant against *A. ochraceus* at high a_w (0.99), but it was not competitive at lower a_w levels (0.95). However, at 30 °C *A. flavus* was dominant at all the a_w levels tested (Lee and Magan, 2000).

Although the use of fungicides is not permitted on maize in Europe, they can be decisively important in the development of *A. flavus* and *A. parasiticus* as *in vitro* studies seem to demonstrate. Criseo et al. (1994) examined *in vitro* the influence of different concentrations of 5 inhibitors of mycelial growth on colony growth by several strains of *A. flavus* and *A. parasiticus*. Cycloheximide and mercuric chloride were the most effective in reducing fungal growth. Dichloran was not able to influence fungal growth, while sodium desoxycholate was. Often with higher concentration of fungicides there was not a higher inhibition of fungal growth. Indeed sometimes, fungal growth was found to recommence after the initial inhibition (Criseo et al., 1994).

More recent studies demonstrated that fungi have a great capacity to adapt to fungicides creating some differences in the colony morphology. Nevertheless, prochloraz and imazalil, two ergosterol biosynthesis inhibitors, seem to be effective in reducing growth of *A. flavus* and *A. parasiticus* (Delen and Tosun, 1999).

1.4.1.6. Aflatoxins production

Years in which AFs contamination is a serious problem are characterized by above-average temperatures and below-average rainfall (Payne, 1998; Payne and Brown, 1998; Scheidegger and Payne, 2005). Field studies managed in the USA showed that in a year with temperatures 2-3 °C higher than normal, high levels of AFs contamination were found; it is difficult to separate the effect of high temperature from that of drought, since they often occur together. However, irrigation has been shown to reduce AFs contamination.

A field study organised in North Carolina showed that the effect of drought stress was most pronounced during the silking to late dough stage of maize grain development. Drought stress likely enhanced infection of kernels and fungal growth (Payne, 1998).

In the field, an important factor to consider is that fungi appear to be simultaneously synthesising and degrading AFs. Consequently, daily environmental changes could distinctly modify the extent of the two metabolic pathways concerned and influence the final level of toxin consequently (Schroeder and Hein, 1968; Stutz and Kruperman, 1976).

In India, the incidence of the toxigenic isolates of *A. flavus* has been checked on different substrates (cereals, dry fruits and spices) and varied considerably, but the highest was found in maize (62 %). In maize strains with the highest AFB_1 production (21 ppm) were always found. The frequency of non-toxigenic strains of *A. flavus* was comparatively higher than the toxigenic ones (ratio 1.07: 1) considering all the substrates of the experiment, but this was not true for maize itself where the ratio non-toxigenic/toxigenic strains resulted 0.61:1 (Bilgrami and Choudhary, 1993).

In a survey conducted in maize fields in Iran, it has been observed that the great part of *A.* section *Flavi* isolated belong to *A. flavus* but, differently from *A. parasiticus* strains that resulted all aflatoxigenic, presented more variability in the incidence of toxigenic strains. Approximately 27.5 % of *A. flavus* isolates were considered as AF producers and 2 of these isolates produced more AFB_2 than AFB_1. In particular, the mean level of AFB_1 produced by *A. flavus* and *A. parasiticus* in this study was in the range 0.08-2.29 μg/ml and 1.85-2.77 μg/ml, respectively (Razzaghi-Abyaneh et al., 2006).

Different results were obtained in a similar study conducted in Italy by Giorni et al. (2007). In this survey, 70 % of *A. flavus* strains isolated from maize fields were able to produce AFs and none of them produced more AFB_2 than AFB_1.

Few studies exist on the different production of AFs by *A. flavus* and *A. parasiticus* in different conditions of a_w and temperature. In an *in vitro* experiment managed growing *A. flavus* strains isolated from maize on artificial media, AFs production was checked in different ecological conditions; three different levels of a_w (0.83, 0.94 and 0.99) and temperature (15 °C, 25 °C and 30 °C) were considered. Only traces of AFs were found at 0.83 a_w while at 0.99 a_w the quantities of AFs produced were the highest. The range of AFB_1 production by *A. flavus* strains was between 0-5 µg/kg at 0.83 a_w, 0-1423 µg/kg at 0.94 a_w and 0-11039 µg/kg at 0.99 a_w with 14 days of incubation. Regarding different temperatures, most strains of *A. flavus* produced the highest quantities of AFB_1 at 25 °C, while at 15 °C and 30 °C the number strains able to produce AFs decreased as did the amount of AFB_1 produced. The range of AFB_1 production was 0-423 µg/kg at 15 °C, 0-2406 µg/kg at 25 °C and 0-505 µg/kg at 30 °C with 14 days of incubation (Giorni et al., 2007).

In a more recent study conducted on *A. parasiticus*, temperatures of 30-35 °C are optimal for AFB_1 production among all the different a_w levels considered (from 0.90 to 0.99 a_w). The minimal temperature to obtain AFB_1 production resulted 17 °C while the maximum resulted 40 °C (Schmidt-Heydt et al., 2010).

Different factors can influence the amount of AFs produced in field; i.e. the competition with other fungi or damages on kernels can produce fungal stress or facilitate fungal growth and, consequently, favour AFs formation (Diener et al., 1987; Lee and Magan, 2000). In an experiment where kernels have been artificially inoculated with *A. flavus* and 13 fungal species normally present on maize, differences in AFs production related to the level of antagonism have been found. When strains were less competitive than *A. flavus* (*Candida guilliermondii, Clodosporium cladosprioides, Curvularia lunata* and *Alternaria alternata*) the AFs contamination was the greatest (mean 1410 µg/kg) while when strains were more competitive than *A. flavus* (*Fusarium moniliforme, Nigrospora oryzae, Acremonium strictum, Penicillium oxalicum, P. funiculosum, P. variabile*) AFs contamination resulted lower (mean 848 µg/kg). When *A. flavus* was paired with *A. niger* or *Trichoderma viride*, no AFs have been detected, probably because these two fungi are able to control the development of *A. flavus* (Diener et al., 1987; Wicklow et al., 1980).

Another important factor that can interact with AFs production in field is the usage of fungicides or chemical treatments. However, a number of *in vitro* studies showed that the use of fungicides at sub-lethal concentrations may enhance mycotoxin production because of the stress caused to the fungus (D'Mello and Macdonald, 1997).

Another important way to reduce AFs is the use of biocompetitive exclusion. Aflatoxin can be degraded by the same species that produce it (Doyle and Marth, 1978); non-aflatoxigenic strains of *A. flavus* and *A. parasiticus* can contribute to AFs degradation. In field trials, Dorner et al. (1999) established that application of non-aflatoxigenic strains of *A. flavus* and *A. parasiticus* greatly altered the overall populations of those species in soil. A 87 % AFs reduction has been measured during the first year of treatment and a 66 % reduction during the following year. It appears that the application of non-toxigenic *A. parasiticus* to soil may not be important in controlling AFs in maize, but the strain of *A. flavus* that is used as a bio-competitive agent is very important.

Some *in vitro* trials established that 9-day-old mycelia of *A. parasiticus* are able to degrade AFs to a varying extent, depending only on the substrate used to grow the fungus. It has been established that aspergilli able to produce greater amounts of AFs are also able to degrade AFs more rapidly while those that produce minimal amounts of AFs generally degraded less effectively (Doyle and Marth, 1978).

In the USA much of the early work on bio-competitive exclusion for AFs management was performed on cotton and AFs contaminated cottonseed was the target for the first atoxigenic strain biopesticide

registration (Cole and Cotty, 1990; Cotty, 1990, 1994). The species most frequently involved in cotton is *A. flavus*. Atoxigenic individuals of this species are frequently isolated from infected crop tissue and the discovery that both ability to infect crops and virulence to crops were not correlated with aflatoxin-producing ability led to the suggestion that atoxigenic strains might be used as biological control agents (BCA) to competitively exclude AFs producers and in so doing reduce the AFs content of treated crops (Cotty, 1989; Cotty, 1992). *A. flavus* communities differed among agricultural fields in aflatoxin-producing potential; application of atoxigenic strains might reduce both the average aflatoxin-producing potential and the vulnerability of all crops planted in those fields to contamination (Cotty, 2006). It has been established that the use of atoxigenic strains of *A. flavus* was able to reduce the average aflatoxin-producing potential of *A. flavus* communities in treated and nearby fields and that these changes to the fungal community persisted for multiple years (Cotty, 1994; Cotty, 2000). In commercial practice, atoxigenic strains are applied on a nutrient source (i.e. wheat seed, barley, sorghum) on which the fungus grows, sporulates and disperses to developing plants and other nutrients in the crop environment (Antilla and Cotty, 2004). Solid formulations allow both residence in treated fields and spore production for relatively long periods and, as a result, provide a window of influence that extents considerably beyond application date (Cotty, 2006).

The use of biocontrol agents has been also considered and suggested by Abbas et al. (2009).

A lot of information is available on this issue but all of them regard studies in abroad countries, in particular in North and South America. This kind of studies can be done taking into account more years, in order to consider also the effect of different weather condition and cultural practices. No studies conducted in Europe are mentioned, probably because in Europe the problem of AFs contamination in maize is new and no studies on more years have been conducted yet.

A summary of the ecological needs of *A.flavus* is given in Table 6.

Table 6: Summary of ecological needs of *A. flavus* reported by different authors

Topic	**Author**	**Range considered**	**T_{min}**	**T_{opt}**	**T_{max}**
TEMPERATURE (T in °C)					
Spore germination	(Ayerst, 1969)	10-50	12	33	43
	(Pitt and Miscamble, 1995)	25-37	25	30	37
Fungal growth	(Ayerst, 1969)	10-50	12	33	43
	(Holmquist et al., 1983)	15-33	15	33	-
	(Pitt and Miscamble, 1995)	25-37	25	30	37
Aflatoxin production	(Giorni et al., 2007)	15-30	15	25	30
Topic	**Author**	**Range considered**	**a_w min**	**a_w opt**	**a_w max**
WATER ACTIVITY (a_w)					
Spore germination	(Ayerst, 1969)	0.70-1	0.78	>0.98	0.99
	(Pitt and Miscamble, 1995)	0.80-1	0.81	0.99	0.99
Fungal growth	(Ayerst, 1969)	0.70-1	0.78	>0.98	0.99
	(Holmquist et al., 1983)	0.85-0.99	0.85	0.99	0.99
	(Pitt and Miscamble, 1995)	0.80-1	0.83	0.97	0.99
Aflatoxin production	(Giorni et al., 2007)	0.83-0.99	0.83	0.99	0.99

1.4.2. Crop management to reduce risks of aflatoxin contamination

Aflatoxin contamination of maize is a complex problem and is influenced by several factors. High temperature, water stress and insects play an important role in AFs contamination as well as other

agronomic factors as tillage system (Jones et al., 1981; Payne et al., 1986). Thus, cultural management coupled with aggressive insect management is current recommended for integrated AFs management (Abbas et al., 2009).

The general strategy is to alter the conditions under which the crop is grown so that infection by the offending fungus or fungi is avoided. Tactics employed include those used to battle most plant diseases: tillage practices, fertilization practices, crop rotation, hybrid choice, planting date and irrigation (Munkvold, 2003).

The development of resistant hybrids appears to be very promising, but commercial hybrids are still not available.

In this section, all the agricultural practices able to reduce or control AFs in maize will be considered one by one.

1.4.2.1. Soil and crop rotation

Crop grown in the previous year have been reported to affect *A. flavus* incidence on the crop in the current year. A 3-year field study revealed that cropping pattern may play an important role in reducing the source of inoculum of *A. flavus*. Soil populations increased threefold in the spring following a maize crop compared with those after a cotton crop. Lowest populations occurred following cotton and wheat crops (Abbas et al., 2004). However, compared to soybean, higher numbers of *Aspergillus* isolates were found in wheat. Therefore, it appears that the use of soybeans rather than wheat in rotation with maize would provide better control of these fungi (Angle et al., 1982).

In a study conducted in Texas (USA), *A. flavus* communities in soils were studied to determine whether crop rotation influence the magnitude and composition of *A. flavus* communities. It has been established that the presence of *A. flavus* (CFU/g) was higher in fields where the previous crop was maize (1,485 CFU/g) compared with either cotton (566 CFU/g) or sorghum (157 CFU/g); moreover, fields previously cropped with cotton had more S strains (28.6 %) than fields previously cropped with maize (17.0 %) (Jaime-Garcia and Cotty, 2006). In this study, several regions of Texas were considered and the trend of results was the same in all of them. Of course, variation in both *A. flavus* population density and strains composition in soils may be influenced by various factors, first of all from variations in weather conditions and soil composition (Jaime-Garcia and Cotty, 2006; Zablotowicz et al., 2007).

Crop rotation is a useful technique to control the spread of potential aflatoxigenic fungi able to survive in crop residues and, consequently, be an important source of inoculum. For this reason, in recent years, in USA there has been a diversification to include maize in a rotation to improve yields, economic returns, reduce pests and improve soil quality (Reddy et al., 2006).

According to Horn (2005), however, there is no short-term effect of crop rotation on soil populations of aflatoxigenic fungi. Soil populations instead depend more on long-term effects of periodic droughts on susceptible crops. In addition to direct dispersal of conidia to soil during harvest, debris from crops can also support colonisation and sporulation of aflatoxigenic fungi once deposited onto the soil (Horn, 2005).

An important role in fungal potential inoculum can be played by the soil texture. In fact, in a study conducted in Mississippi Delta soils, populations of *A. flavus* were highest in soils with the greatest organic matter content (Zablotowicz et al., 2007) confirming results obtained previously (Klich, 2002; Wicklow et al., 1984). However, this study took into account two years (2000 and 2001) and results in the two years were different, probably due to different weather conditions. In particular, in 2000,

aflatoxigenic isolates recovered from the various soils collected in this area ranged from 31 to 75 % with a similar range found in soils with no maize history and soils with a history of maize cultivation (Zablotowicz et al., 2007). In 2001, there were more soils evaluated and the range of maize history was from zero to 4 years in the previous decade. Results obtained underlined that the populations of *A. flavus* collected from soils were highly correlated with the years of maize cultivation. The highest propagule densities of *A. flavus* were associated with soils having the greatest fertility; thus, the greatest potential for AFs contamination of maize by soil inoculum of *A. flavus* may be associated with some of the more productive soils (Zablotowicz et al., 2007). Moreover, soils with high organic matter have a greater moisture-holding capacity than soils with a lower organic matter content resulting in a reduced potential for moisture stress and susceptibility to AFs contamination in crops grown in soils with a high organic matter content (Zablotowicz et al., 2007).

From the literature it is stressed that soil composition and weather conditions can influence the amount of *A.* section *Flavi* inoculum concentration in soil. These aspects can be taken into account in order to define the risk level for AFs contamination in grain, but they cannot be modified. Crop rotation, which is a choice of farmers, is also relevant, apparently crucial, and in high risk condition it has to be applied to reduce the risk, even if its limited effect in short term was highlighted.

1.4.2.2. Tillage system

Another agronomic factor that is reported to affect *A. flavus* concentration is tillage. Tillage practices range from no-tillage to plowing and cultivating the soil; they directly influence the physical and chemical properties of the soil, root growth, nutrient uptake and population of other soil microorganisms, which ultimately affect the viability and activity of plant pathogens and the susceptibility or resistance of the host. Reduced tillage can favour plant pathogens in general by lowering soil temperature, increasing soil moisture, changing root growth and nutrient uptake (Singh and Sharma, 2002). However, it is unclear whether this also accounts for *Aspergillus* spp. as there are conflicting results on this pathogen.

A study conducted for 6 years in Mississippi (USA), demonstrated that maize produces higher levels of plant residues in comparison to other crops. For this reason, adoption of no-tillage practices generally increases soil organic matter in the surface soil (Locke et al., 2005; Reeves, 1997). The high amount of maize residues and their particularly high stability and persistence can increase the presence of soil organic carbon resulting in reduced soil erosion and improvement of soil fertility (Reddy et al., 2006). *A. flavus* populations increased in no-tillage fields compared with adjacent conventionally tilled soils (Reddy et al., 2007). However, in a previous study, similar populations of *A. flavus* were observed under conventionally tilled and no-tilled soils (McGee et al., 1996). Another study in maize reported a lower percentage of *A. flavus* and *A. parasiticus* in the soil under no-till cultivation as compared to conventional tillage (plowing and disking) (Angle et al., 1982). Others hardly found any difference between conventional and no-till practices in wheat for various fungi (including *A. flavus*) (Ahmad and Iram, 2008).

The basic assumption is that AFs levels are greater during years when drought stress occurs and any procedure that alleviates drought stress should result in lower levels of AFs. Both tillage and subsoiling are techniques able to reduce stress and lead to reduced contamination with AFs. In particular, in a 4-year study conducted in North Carolina (USA), subsoiling and conventional tillage were able to reduce AFs contamination; subsoiling resulted to be the best treatment able to reduce AFs contamination of 55 % respect to conventional tillage in one year (1982) while conventional tillage resulted better in 1983 (warmer and drier) when obtained 29 % less contamination than subsoiling (Payne et al., 1986); differences in years can be partially affected by the different level of fungal inoculum present in field.

Tillage is considered one of the best ways to reduce fungal inoculum in field. In fact, tillage removes plant debris, where fungi can easily survive and sporulate, and results in a lower presence of fungal spores in the upper layer of soil and in higher difficulties for fungal population to have ecological conditions necessary to sporulate and contaminate maize plants. Nevertheless, reported studies do not allow conclusive remarks being sometimes in contrast. In particular, it is not possible to quantify the effect of tillage system on AFs contamination.

1.4.2.3. Cultivar and hybrid

The choice of maize hybrids is one of the most important in the battle against AFs contamination.

In many regions, as Latin America, Africa and southern Asia, where maize is cultivated, limited financial resources impede the purchase and use of hybrid. As a result maize cultivars grown in these countries are often open-pollinated types or blends, which lack the heterosis of hybrids. This determine no genetic propensity to tolerate drought stress and, consequently, their higher susceptibility to fungal infection and AFs contamination (Bruns, 2003). Also some surveys reported that open pollinated cultivars of maize commonly grown in USA prior to extensive use of hybrids, were more susceptible to pre-harvest AFs contamination than hybrids (Zuber et al., 1987).

Different substances and compounds present in maize have been identified and studied as possible use to increase genetically resistance to *A. flavus* (Bruns, 2003). For example, the enzyme β-1-3-Glucanase, when present in maize kernels, has been suggested to have a role in the inhibition of *A. flavus* growth on the grain (Lozovaya et al., 1998).

Some proteins have been also studied to increase resistance against fungal and mycotoxin infection. In particular, in the resistant maize inbred Tex6 have been found two interesting proteins: one with a mass greater than 100 kDa, that inhibits AFs production with no effect on fungal growth while the other one, with a mass of 28 kDa, inhibits the growth of *A. flavus* (Huang et al., 1997). Important was the discovery of a 14 kDa trypsin inhibitor always found in high concentrations in maize genotypes that were resistant to both *A. flavus* and *A. parasiticus*, and at low concentrations, in susceptible genotypes (Chen et al., 1998).

Resistance can also be structural, i.e. it can be due to kernel pericarp wax and husk covering over the ear. Wax and cutin layers on maize kernels may play a role in resistance to AFs accumulation in certain genotypes (Guo et al., 1995). It has been shown that the resistant genotype had an abundant amount of wax deposits on the kernel surfaces while the susceptible hybrids did not (Russin et al., 1997).

Some of the first researches on maize resistance to *A. flavus* infection and AFs contamination involved the indirect protection of developing kernels by long tight husk coverings which helps in reducing the feeding of insects, an aid in the infection process (Lisker and Lillehoj, 1991; McMillian et al., 1985).

Despite these discoveries, little of this genetic material is being publicly advertised as being mycotoxin resistant because its effectiveness may be limited by environmental conditions in the field. In any case, genetically resistant material will require good crop management practices to be employed in growing the crop for full resistance to be realized and mycotoxin contamination prevented (Bruns, 2003).

Of course, the choice of hybrid has to be done taking into account both water availability in the area where maize is cultivated and the possible harvest date. In Italy, several survey conducted in Emilia Romagna, underlined that early hybrids (FAO class 300-400) are the most prone to AFs contamination since they complete grain maturation in a period of high temperatures and poor precipitation (Battilani, 2004).

It has been recently stated that the dynamic of reduction in both a_w and humidity in maize kernels during ripening is different in diverse hybrids, but this is not related to the FAO class, as previously stated by several authors. The water content of kernels during ripening influences the production of fumonisins and "slow dry down" hybrids, irrespective of their season length, were confirmed as more prone to fumonisin accumulation; both a_w and humidity of kernels at harvest in ten maize hybrids have been shown as accurate predictors of contamination (Battilani et al., published on line). It is expected this approach can give interesting results also for AFs prediction, being the synthesis of these toxins favoured with kernels humidity lower than 32 %.

In a study conducted in USA, both susceptible and resistant hybrids were artificially contaminated and used to evaluate the presence of *A. flavus* along the growing season. The percentage of infected grains was similar in resistant and susceptible hybrids after, respectively, 46 and 50 days after midsilk; however, the percentage of infected grains was greater in susceptible hybrids at later harvest date (Scott and Zummo, 1994). Thus, resistant hybrids differed significantly from susceptible hybrids for infection levels at harvest dates of 54-62 days after midsilk concluding that the selection for resistance to *A. flavus* should be more effective at harvest dates around 60 days after midsilk than when grain reaches physiological maturity (Scott and Zummo, 1994).

The choice of maize hybrid is probably the most important for AFs control. Bt-maize could be a valid option to reduce insect damages and, consequently, fungal infection. Breeding and selection of hybrids less susceptible to *A. flavus* and *A. parasiticus* attack are under study. The structural resistance to infection (composition of pericarp wax or husk particularly protective for ears) has been the most considered, till now. Breeding for resistance is ongoing, but resistant hybrids are not yet available on the market.

1.4.2.4. Seeding

Temperature is probably the most important environmental factor influencing pre-harvest infection of maize by *A. flavus* and the eventual contamination of grain by AFs (Fortnum, 1986; Jones et al., 1980). As temperatures increase, AFs contamination also increase (Manwiller and Fortnum, 1979; Williams et al., 2003); of course, nothing can be done to control ambient temperatures, but it is possible to avoid their impact during the later stages of kernel filling by early planting (Abbas et al., 2009). A research conducted in North Carolina (USA) demonstrated that lower levels of AFB_1 contamination occurred in maize grain produced by April compared to May seeding (Jones and Duncan, 1981; Jones et al., 1981). In other studies conducted in Florida and Georgia (USA), a higher incidence of AFB_1 was observed in June-planted maize in comparison to April and May plantings (Lillehoj et al., 1978).

Seeding time seems to be of high importance among events leading to infection, so a change in seeding date can significantly affect mycotoxin accumulation. In maize, earlier planting dates in temperate areas generally result in a lower risk, however annual differences in weather conditions can limit this advantage (Munkvold, 2003).

However, in another geographic area and, above all, in a different year, things could be different. For example, in a study conducted in the Coastal Plain of Georgia, early April maize seeding resulted at greater risk to AFs contamination than mid-May or later seeding (Widstrom et al., 1990). Probably this happened because the critical period in kernel filling, that begins approximately 20 days after anthesis, occurred when seasonal maximum and minimum temperatures were highest creating the most favourable environmental conditions for *A. flavus* development in field and, consequently, AFs production (Widstrom et al., 1990). A survey in this same region conducted years earlier (in 1978) showed that grains harvested in September presented lower levels of AFs than those harvested in July (McMillian et al., 1980); this was probably due to different weather conditions registered in different years.

However, it seems that maize that experienced 41 days of ambient temperatures > 32 °C during kernel filling had greater levels of AFs contamination than those produced in subsequent years that had 30 days of ambient temperatures > 32 °C, regardless of planting date or maturity rating (Bruns and Abbas, 2006).

Probably, the efficacy of early planting is based on the moving of the period between anthesis and dough-development in maize to a time frame in the growing season where drought and heat stress are less likely to be encountered, especially in comparison to later plantings (Zuber et al., 1976). Another possible effect of early seeding is a better development of maize roots that can favour plant ability to resist to water stress (Roth et al., 1995).

Based on the literature, early seeding seems a suggested practice to reduce the risk of AFs contamination in maize, but published data are very variable and strongly influenced by years and growing conditions; besides, no quantitative data are available on the effect of seeding period on AFs contamination in maize. It is not surprising because seeding time indirectly affect the risk of AFs contamination, both moving the growth stages in different periods of the year and giving a different opportunity to plants to develop a good root system. Above all, early seeding is probably a risk–reducing practice, but it is clear that a quantification of seeding time on AFs contamination will never be possible being involved in the final result many factors strongly variable in different areas and years.

1.4.2.5. Irrigation

As previously described, drought and high ambient temperatures are known to favour *A. flavus* growth and AFs production. Approximately 90 % of the maize grown for grain in South Carolina (USA) in 1977 and 1978 was contaminated with AFs due to drought and heat stress (Jones et al., 1981; Manwiller and Fortnum, 1979).

Drought stress can occur in maize rather quickly if exposed to a combination of high ambient temperatures and low relative humidity (Abbas et al., 2009) and, for example, in southeast of USA, high heat is generally accompanied by low rainfall or drought. In short season maize hybrids, yield reduction of more than 50 % can occur when brief periods of wilting are registered at 50 % silking (Denmead and Shaw, 1960; Robins and Domingo, 1953). However, maize hybrids produced in the last 30 years seem to be more tolerant to drought stress than those used in earlier years (Duvick, 1984) and they do not suffer the adverse effects of drought as soon as older hybrids did (Abbas et al., 2009).

When maize can be irrigated, drought stress is reduced and AFs levels are lower (Abbas et al., 2009). In a study conducted in North Carolina (USA), a reduction in *A. flavus* infection and AFs contamination was observed in irrigated *versus* non-irrigated maize. These differences were observed also in years when rainfall is below normal quantity (Jones et al., 1981). In particular, in a two-year study conducted in 1978-1979, irrigated plots contained 23.6 % AF-positive samples compared to 54.9 % positive samples in non-irrigated plots and the effect of irrigation on levels of AFB_1 was really relevant. In particular, irrigated subplots contained an average of 7.3 µg/kg compared to 61.9 µg/kg in non-irrigated subplots. Moreover, irrigated subplots contained fewer visibly infected ears and had higher yields (Jones et al., 1981).

Another study conducted in 1982 on maize grown in the same area confirmed that AF levels in the non-irrigated fields were significantly higher than in all of the irrigated ones (Payne et al., 1986).

In a study conducted in two Egyptian regions (Nubaria and Sakha) on different maize genotypes under surface and sprinkler irrigation, it was found that the type of irrigation used can influence mycotoxins content in grains. In particular, in this study, an higher *A. flavus* infection was observed under surface irrigation system (Tolba et al., 2005).

It is also important to remember that irrigation improves the biological value of maize protein and reduce kernel breakage susceptibility (Mason and D'Croz-Mason, 2002) and, indirectly, it is a good way to control fungal and AFs contamination.

In areas where irrigation is not applied because of water availability or costs, early seeding and drought stress adapted hybrids are suggested (Miller, 2001).

Irrigation has been definitely recognised to play an important role on AFs control in maize. Depending on the geographic area, it is not always applicable both for unavailability of water or for excessive costs. In these cases, literature suggests to use different agricultural practices to reduce the impact of lack of irrigation. In particular, early seeding and hybrids able to better resist to drought stress are suggested. Also regarding irrigation, quantitative data on its effect are not available and very difficult to obtain because of the number of factors involved, like kind and timing of irrigation, water volume distributed, water deficit in plants, and their interaction with weather conditions.

1.4.2.6. Fertilisation

A rationale management of fertilisation is crucial to avoid plant stress, intended as deficiency or excess of nutrients, because of their effect on AFs production. Adequate plant nutrition, and in particular sufficient levels of nitrogen is necessary to prevent AFs contamination of preharvest maize grain (Jones, 1979; Lillehoj and Zuber, 1974).

Nitrogen (N) is the most relevant compound, it is both relevant for plant metabolism and it has a significant effect on plant vigour, that influence the microclimate in the canopy and the maize-*A. flavus* interaction. Nitrogen is the most important element in structural and metabolic proteins as well as nucleic acids. Consequently maize plants suffering from N deficiencies during grain developing introduce this element in an alternative way, first of all translocating N from older leaf tissue to the developing grain and eventually abort the older leaves (Bruns, 2003).

Nitrogen deficiencies can arose from drought stress that is able to alter the uptake and translocation of N in maize (Younis et al., 1965) or from a wrong and underestimated fertilization. In this way, plant stress resulting from low N-ferilisation rates determines the increment of AFs presence in maize (Lillehoj and Zuber, 1974). In particular, insufficient levels of mineralized N in the root zone can be due to either drought stress or to excessive rain and, once again, predispose maize to AFs contamination (Jones, 1979)

A confirmation of N fertilization importance comes from a study conducted in USA where higher levels of AFs contamination occurred in maize grain produced with 80 kg/ha of N compared with that produced with 120 kg/ha (Anderson et al., 1975). In another study managed in USA, maize grown with low levels of N-fertility (11.2 kg/ha) had consistently higher AFs levels than grain produced with high N-fertility rates (145.7 kg/ha) (Jones and Duncan, 1981). In particular, the low N-fertility treatments had 2.4 times more AFB_1 than the high N-fertility treatments (2000 *versus* 4875 ng/g) (Jones and Duncan, 1981).

However, an inadequacy of any nutrient element (like phosphorus, potassium, and calcium) has the possibility to increase plant's susceptibility to attack by most all forms of plant pathogens (Stromberg et al., 1999).

Fertilisation can modify plant development and cause relevant plant stress, if not well balanced. Therefore, a fertilisation based on plant needs is crucial to avoid conducive conditions for AFs. The quantification of unbalanced fertilisation on AFs production is very difficult because data published report N distributed, but the reference to soil content is not included; the balance between N available and plant needs is not possible. Nevertheless, a balanced fertilisation is part of good agricultural

practices (GAP) and this aspect must be mandatory for farmers irrespective of AFs risk in the maize growing area.

1.4.2.7. Weeds control

Maize crops harvested from weed-infested fields usually present yield reductions and a decreased grain quality similar to those harvested from drought stressed fields (Rice, 1984). The presence of weeds in maize crops can indirectly cause a higher incidence of *A. flavus* and AFs in grains. This is because they can induce stress to maize plants due to their competition for water, nutrients and sunlight (Bruns, 2003) causing deleterious effects on yield. Moreover, certain weed species are also able to exude chemicals, from their roots into the soil, that inhibit crop development, a process known as allelopathy (Rice, 1984).

In several studies it has been stated that the weed canopy in maize contributes to the contamination of grain by AFs due to the stress they exert on the crop (Cobb, 1979; Lillehoj, 1983); heavier is the weed infestation in maize and higher will be the stress they cause to maize.

Weeds have to be reduced to indirectly control fungal presence in field. In fact, they result to be able to compete with maize plants for nutrients and water. This leads to plant stress and, consequently, in a higher susceptibility to *A. flavus* attack in case environmental conditions are conducive. Nevertheless, weeds control is also part of GAP and mandatory for farmers, as stated for fertilisation.

1.4.2.8. Pest control

One of the recognised factors to enhance AFs contamination in preharvest maize is insect damage of grains (Beti et al., 1995); insects act as vectors, facilitating spore entry into ears and increasing infection by damaging the kernel pericarp (Lillehoj et al., 1978; Payne, 1992; Widstrom, 1979).

In a study conducted in Benin, maize ears with less than 2 % insect feeding damage had a mean AFs contamination level considerably lower than ears with more than 10 % damage in both years of observation (Setamou et al., 1997).

In an earlier survey conducted in Missouri and Illinois (USA) in 70s, maize ears that had been extensively damaged by ECB (*O. nubilalis*) and corn earworm (*Heliothis zea*) had significantly higher levels of AFs than undamaged ears (Lillehoj et al., 1975). From this study arose also that insects vectoring of the fungal inoculum occurred due to larvae ingesting spores and transferring the infection to developing kernels through their frass (Lillehoj et al., 1975).

In a study conducted in northern Mexico, a higher incidence of ears with insect damage was observed in fields without insecticide applications but, in some cases, insects increased also in thesis under high plant density, drought stress and late planting, all of which received insecticide applications (Rodriguez-del-Bosque, 1996). In particular, a combination of good cultural practices (early planting, reduced plant population and irrigation), together with an optimal maize hybrid and insect control, reduced AFs concentrations down to 0-6 ng/g, compared to 63-167 ng/g in late-planted, non irrigated maize at a higher plant population without insect control (Rodriguez-del-Bosque, 1996).

Generally, the control against ear feeding insects is limited to high-cash value food maize such as sweet maize or white maize while yellow maize grown for livestock feed is seldom treated with insecticides (Bruns, 2003). It has to be considered that chemical treatments are usually expensive and, in addition to this, it is necessary, especially to control ECB, to determine the stage of the insect's lifecycle and timing the application of insecticide to optimise the control. Late treatment of infested fields will be ineffective once the borer enters the interior of the stalk (Bruns, 2003). In recent studies managed in Italy it was suggested to schedule ECB control as silk browning instead of based on the

insect detection in field. It seems to generate good control even limiting time for field surveys (Mazzoni et al., submitted).

Regarding corn earworms, instead, numerous insecticide applications are required to easily control it but this make the cost of applications prohibitive for feed grain (Bruns, 2003). Nevertheless, this is not a relevant problem in Europe.

The most successful approach has been the use of maize resistant to ear-feeding insects. Several authors have shown that *Bacillus turingensis* (Bt)-transformed maize hybrids, which are resistant to ear-feeding insects, reduce AFs contamination of the grain (Dowd, 2000; Munkvold et al., 1999; Williams et al., 2002). Comparing non-Bt with Bt maize hybrids, it has been demonstrated that corn borer larval establishment was significantly higher on the firsts. Larval survival was extremely low on Bt hybrids indicating that these hybrids should be effective in reducing AFs contamination in areas where high corn borer infestations occur (Williams et al., 2005). The adoption of Bt maize hybrids has given producers a crop with increased insect resistance, however these hybrids may only reduce AFs contamination under certain circumstances (Abbas et al., 2009) and they are not admitted in most European countries.

Damages caused by insects are well known to be conducive of *A.* section *Flavi* infection, not only for grain cracking but also through the movement of insects that can be, with their body or frass, vectors of fungal inoculum. A good management of maize field has to consider pest control to reduce AFs contamination of grains.

1.4.2.9. Aspergillus section Flavi control

No fungicides are actually registered for maize in Europe and data available only result from *in vitro* trials.

Early investigation *in vitro* indicated that the fungicide chlobenthiazone is highly effective in inhibiting AFs biosynthesis by *A. flavus*; however AFs synthesis by *A. parasiticus* was stimulated by this fungicide (Wheeler et al., 1991).

Some possibilities to contrast these fungi come from various surfactants, including some used in pesticide formulations, that resulted able to reduce AFs biosynthesis by more than 96 % (Rodriguez and Mahoney, 1994). The use of some natural compounds had also good results, in particular natural oils from thyme (Kumar et al., 2008), lemongrass (Bankole and Mabekoje, 2004) and other herbs have been studied to repress AFs content in certain crops in Asia, but they were not tested on maize.

Positive results obtained *in vitro* were not supported by field trials; in fact, conventional methods of plant disease control with the use of fungicides and insecticides were ineffective in controlling *A. flavus* infection of maize when employed at concentrations that are both cost-effective and environmentally safe (Bhatnagar et al., 1993).

Actually, agronomical strategies and the use of biocontrol agents are the only methodologies effective to contrast these two aflatoxigenic fungi.

A summary of the possible techniques to reduse AFs contamination in maize is given in Table 7.

Table 7: Summary table of all the known possible techniques to reduce AFs contamination in maize with crop, insect and soil management found in the papers considered (Abbas et al., 2009, modified).

Strategy	Method	Rationale
Avoidance	Early planting, irrigation	Reduce heat and moisture stress
Fertility management	Provide adequate nutrition	N-deficient maize more susceptible
Insecticide application	Appropriate timing of product application	Reduce damage to ears from insects
Hybrid choice	Bt-hybrids or hybrids with natural resistance to insects or drought stress	Increase resistance of ears to insects damage and water stress.
Biological control	Use of nontoxigenic isolates of *A. flavus*	Reduction of toxigenic isolates thanks to in field biocompetitive exclusion
Soil management	Incorporation of crop residues with tillage	Reduce inoculum density

Several data have been published on the effect of fungicides on *A. flavus* and *A. parasiticus in vitro*, but no specific commercial products are available on the market. An important consideration, when the use of fungicide is decided, is to evaluate the possible effect on fungal population. Since a higher AFs production is linked to a stress status of the fungus, it is necessary to be sure that the ingredient used is effective both on *A. flavus* and *A. parasiticus* to avoid contributing to more conducive conditions. Reported results are not conclusive, regarding the possible use of fungicides to mitigate AFs production.

1.4.3. Modelling Aspergillus section Flavi and its interaction with crops

Modelling is always a useful approach in complex systems as a support in the prediction of risk level in different ecological conditions and possibly under diverse cropping systems. Nevertheless, few publication are available regarding modelling of *A.* section *Flavi*.

1.4.3.1. Modelling *Aspergillus flavus* in different ecological conditions

Aspergillus flavus growth and AFs production after different incubation times was studied on artificial media (potato dextrose agar-PDA; 28 °C in the dark) *in vitro* and on maize ears in field with the aim of finding a simple model able to describe the results (Abbas et al., 2008). The Gompertz model was found as useful to describe growth parameters, intended as growth constant, lag phase and maximum colonisation. The same model described well AFs accumulation *in vitro* and in field, except with very low mycotoxin production, better described by linear or logistic regression.

Aspergillus flavus and *A. parasiticus* growth was modelled based on water activity regimes during growth. After the transformation of a_w values, a quadratic function was applied and the coefficient found proved to be useful for the species considered and applicable independently on the fungal strain (Baranyi et al., 1997; Gibson et al., 1994).

A simple model, defined from cardinal values of environmental factors (minimum, optimum and maximum values; Rosso type cardinal model family), was proposed to describe the effect of water activity on the radial growth rate of several moulds, *A. flavus* and *A. parasiticus* included. The results showed a good concordance between the predicted and the observed values for these species (Rosso and Robinson, 2001). A similar approach was followed by other authors and they confirmed the reliability of results (Sautour et al., 2001).

The growth of *A. flavus* and *A. parasiticus*, artificially inoculated in maize kernels in different temperature (16 °C, 22 °C, 25 °C, 30 °C and 37 °C) and water activity (between 0.801 and 0.982) regimes, was studied with the aim of modelling the effect of both factors (Samapundo et al., 2007). The colony growth rates (GR) and lag phases (λ) were estimated by fitting a flexible primary growth model. Subsequently, secondary models relating GR or λ to a_w, temperature or a_w and temperature combined, were developed and validated. The Gibson and linear Arrhenius–Davey model describing the individual effects of a_w or temperature on GR or λ gave adequate predictions. A quadratic polynomial function proved to be the best predictors of the combined effect of the parameters. The estimated T_{opt} and the observed a_{wmin} for growth were in good agreement with those reported in previous papers; T_{opt} was approximately 30 °C and no growth was observed at a_w 0.801, growth only occurred at 25 and 30 °C at a_w 0.822.

A modelling approach was also followed by Pitt (1993) to integrate the effect of temperature, water activity, pH and *Aspergillus* colony size on mould growth and AFs production. The rate of toxin production was assumed to be proportional to the rate of production of new cell mass and it was correctly predicted as related to temperature and temperature cycling.

Several authors applied modelling to predict fungal behaviour, but knowledge is very limited regarding AFs production. Besides, these simple models are interesting but they need to be integrated in a more complex system approach to be used at field level.

1.4.3.2. Modelling *Aspergillus flavus* – maize interaction

Modelling the interactions between host plant and environment during the season can enable prediction of pre-harvest AFs risk and its potential management.

A simulation model to predict AFs contamination in maize was developed using a module which was previously developed for peanuts, using the APSIM peanut model. The model was further applied in combination with long term weather records to examine the effects of agricultural factors such as sowing date, plant population, nitrogen supply, and cultivar duration on pre-harvest aflatoxin risk in a probabilistic framework. According to the authors, the module has to be fully validated for maize; then, it will be possible to provide growers and industry personnel with both pre-season and in-season probabilities for AFs risk (Chauhan et al., 2008). They also approached a scenario analysis and they suggested that under non-irrigated conditions the risk of AFs contamination could be minimised adjusting sowing time or selecting appropriate hybrids to better match the grain filling period with lower temperature and water stress conditions.

A simple approach was applied to Italian data in order to define the risk to harvest grains above the European legal limit of 5 µg/kg. The aridity index (AI) was computed on a 10-day base from June to September, to summarise meteorological conditions. The AIs were used as independent variable to run a logistic regression with the aim of estimating the probability of AFB_1 contamination in maize at harvest. The logistic regression gave reasonable warning on AFB_1 contamination in maize with 64 % correct predictions, 23 % overestimates and 13 % underestimates. First indications with this simple predictive system are available before mid July with conclusive information in early September, which is a good time to plan maize pre- and post-harvest management (Battilani et al., 2008b).

1.5. Climate change

1.5.1. Climate change: impact and adaptation

The physics and chemistry of the Earth's atmosphere largely determines our climate. Although the atmosphere seems like a huge reservoir capable of absorbing almost limitless quantities of our industrial emissions, it is really only a thin film. Our understanding of how the chemistry and physics of the atmosphere affect climate developed over many centuries. In 1859, Tyndall suggested that water

vapour, CO_2 and other radioactively active ingredients could contribute to keeping the Earth warm, but our understanding has greatly accelerated during the past few decades.

In 1988, the World Meteorological Organization and the United Nations Environment Program jointly established the Intergovernmental Panel on Climate Change (IPCC). The IPCC consists of a set of committees of leading scientists from all around the world whose task is to periodically review and report on the state of understanding of the climate problem.

The 2007 assessment of IPCC drew two substantially new conclusions which have had a marked effect on policymakers. The first was that current climate change is "unequivocal" and is due largely to emissions of greenhouse gases resulting from human activity. The second was that the effects of this observed global warming can now be detected on every continent in the form of altered hydrology and biology.

Climate change may directly, but also indirectly, affect fungal infection. It may directly influence host susceptibility through heat and drought stress. Indirectly it affects the planting time of the crops. When patterns change, even well planned crops may become exposed to conditions favourable to contamination. Climate also affects fungal species present on crops where *Aspergillus* growth is favoured by warm and dry conditions. Furthermore, climate influences the extent to which crops become wounded by mammals, birds and insects, which can act as vectors for *A. flavus* and *A. parasiticus* on rice plants.

Climate change, involving changes in mean climate and climatic variability, is expected to severely affect agriculture and there is a need to assess its impact in order to define the appropriate adaptation strategies to cope with. The results of recent global climate monitoring as well as climate projections stress that future climate will be significantly different than that experienced in the past (Rosenzweig et al., 2007). These changes are expected to affect many economic sectors, including agriculture, forestry, energy consumption and tourism (Hanson et al., 2007).

Since agricultural practices are climate-dependent and crops growth and yield vary from year to year depending on the weather, the agricultural sector is particularly exposed to climatic change. Understanding the potential impacts on agriculture of a warming climate has thus become increasingly important and it is of primary concern particularly to ensure the sustainability of agricultural systems as well as for policy-making purposes (Howden et al., 2007). In order to predict the climate variation in future scenarios, modelling is considered the suitable approach.

1.5.1.1. Global Climate Models

Global Climate Models (GCMs) use mathematics to describe how the atmosphere, the oceans, the land, living things, ice, and energy from the sun affect each other and Earth's climate. Thousands of climate researchers use global climate models to better understand how global changes, such as increasing greenhouses gases or decreasing Arctic sea ice, will affect the Earth. The models are used to look hundreds of years into the future, so that it can be predicted that our planet's climate will likely change. There are various types of climate models. Some focus on factors that affect climate such as the atmosphere or the oceans. Models that look at few variables of the climate system may be simple enough to run on a personal computer. Other models take into account many factors of the atmosphere, biosphere, geosphere, hydrosphere, and cryosphere to model the entire Earth system. They take into account the interactions and feedbacks between these different parts of the planet.

Earth is a complex place and consequently many of these models are very complex too. They include so many mathematic calculations that they must be run on supercomputers, which can do the calculations quickly. All climate models must make some assumptions about how the Earth works, but in general, the more complex a model is, the more factors it takes into account, and the fewer

assumptions it makes. At the National Centre for Atmospheric Research (NCAR, Bouleder Colorado), researchers work with complex models of the Earth's climate system. Their Community Climate System Model is so complex that it requires about three trillion mathematic calculations to simulate a single day on planet Earth. It can take thousands of hours for the supercomputer to run the model. The model output, typically many gigabytes large, is analysed by researchers and compared with other model results and with observations and measurement data. There are currently several other complex GCMs that are used to predict future climatic change. The most robust models were compared by the Intergovernmental IPCC as they summarize predictions about future climate change (Figure 9).

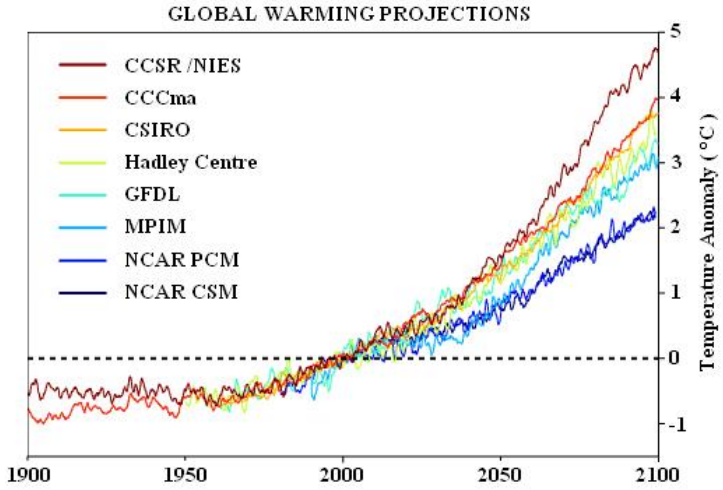

Figure 9: IPCC graphic of uncertainty ranges with various models over time (GNU, on line)

1.5.1.2. Lars weather generator

The output from GCMs is of insufficient spatial and temporal resolution and reliability to be used directly in impact models. A stochastic weather generator can serve as a computationally inexpensive tool to produce multiple-year climate change scenarios at the daily time scale which incorporate changes in both mean climate and in climate variability (Semenov and Brooks, 1999)

A downscaling procedure has been set up in order to reproduce, on a scale suitable for impact assessment in agriculture i.e. 50 × 50 km, the future climate at an average global warming of + 2 °C and + 5 °C, above preindustrial levels. Two emission scenarios are taken into account in order to evaluate minimum expected change, resulting in a low increase in global temperature, and maximum expected scenarios, with temperature increases of + 5 °C. It should be noted that the + 2 °C scenario for the time horizon 2100 is generally considered a very ambitious target (Moriondo et al., 2010), underestimating what could be the evolution. It will require very effective climate change mitigation (to curb the growth of atmospheric concentrations of greenhouse gases), undertaken from early on. If atmospheric concentrations of greenhouse gases grow in the business-as-usual mode, i.e. no effective mitigation is in place, commencing in the near future, the increase in average temperature by 2100 is likely to be much higher, e.g. at the level of + 4 °C (Moriondo et al., 2010).

This procedure was based on the use of the LARS weather generator (LARS WG) weather generator that allowed including changes in mean climate as well as in climate variability as derived from a GCM in future climate simulations.

LARS-WG is a stochastic weather generator which can be used for the simulation of weather data at a single site (Racsko et al., 1991; Semenov and Barrow, 1997) under both current and future climate conditions. These data are in the form of daily time-series for a suite of climate variables: precipitation (mm), maximum and minimum T (°C), solar radiation ($MJm^{-2}day^{-1}$), RH (%).

Stochastic weather generators were originally developed for two main purposes:

(i) To provide a means of simulating synthetic weather time-series with statistical characteristics corresponding to the observed statistics at a site, but which were long enough to be used in an assessment of risk in hydrological or agricultural applications.

(ii) To provide a means of extending the simulation of weather time-series to unobserved locations, through the interpolation of the weather generator parameters obtained from running the models at neighbouring sites.

Stochastic weather generator is not a predictive tool that can be used in weather forecasting, but it is simply a means of generating time-series of synthetic weather statistically 'identical' to the observations.

1.5.1.3. Model implementation

IPCC published a set of emissions scenarios in 2000 for use in climate change studies (Special Report on Emissions Scenarios – SRES). The SRES scenarios were constructed to explore future developments in the global environment with special reference to the production of greenhouse gases and aerosol precursor emissions. The SRES team defined four narrative storylines, labelled A1, A2, B1 and B2, describing the relationships between the forces driving greenhouse gas and aerosol emissions and their evolution during the 21st century for large world regions and globally. Each storyline represents different demographic, social, economic, technological, and environmental developments that diverge in increasingly irreversible ways:

A1 storyline and scenario family: a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter, and rapid introduction of new and more efficient technologies.

A2 storyline and scenario family: a very heterogeneous world with continuously increasing global population and regionally oriented economic growth that is more fragmented and slower than in other storylines.

B1 storyline and scenario family: a convergent world with the same global population as in the A1 storyline, but with rapid changes in economic structures toward a service and information economy, with reductions in materials intensity, and the introduction of clean and resource efficient technologies.

B2 storyline and scenario family: a world in which the emphasis is on local solutions to economic, social, and environmental sustainability, with continuously increasing population (lower than A2) and intermediate economic development.

All scenarios were designated as equally valid, with no assigned probabilities of occurrence.

2. MATERIALS AND METHODS

2.1. Literature search

The inventory of literature was based on the scientific literature and official reports of well recognised organisations and national and international projects, following the principles of the systematic literature review (EFSA, 2011).

The main sources of information were CAB abstracts on OVIDSP; ISI Web of Sciences and Scopus. In these big databases it is possible to find all papers published from 1978 to today. In particular:

OVIDSP offers a premier selection of the world's most respected databases across a wide range of disciplines, counting hundreds of top databases, more than 1500 scientific journals and international electronic books, links to full text articles in Journals@Ovid and Kluwer Journals, or external sources thanks to full text linking technologies;

Scopus has nearly 18,000 titles from more than 5,000 international publishers, including coverage of 16,500 peer-reviewed journals (including over 1,200 Open Access journals); 600 trade publications; 350 book series; Extensive conference coverage (3.6 million conference papers). It has 38 million records, of which 19 million records including references going back to 1996 (78 % include references), and 19 million pre-1996 records going back as far as 1823.

A specific research for reports on aflatoxin in cereals was run in Internet, both using Google and visiting specific sites managing mycotoxin issues.

The main objectives of the review question are as follows:

(1) *Aspergillus* spp in wheat, in maize and in rice; (2) Crop growth models for wheat, for maize and for rice; (3) Climate change; (4) Aflatoxin occurrence; (5) Crop management for maize; (6) Modelling *A.* section *flavi* in cereals crop.

The search terms used are reported in Appendices A-J.

Depending on additional information needed, further key words were added during reporting:

Inoculum, overwintering, sclerotia, spore dispersal, strain, nutritional sources, weather, growing area, growth stages, silk emergence, plant water stress, agronomical factors, irrigation, manuring, fertilisation, nitrogen, crop rotation, soil tillage, crop variety, crop resistance, sowing date, flowering, ripening, harvest, crop residue, interaction.

When information on a specific issue was lacking, different key words were tested to better focus the research. The tentative of having a fully complete literature, including all possible key words, produced an enormous list of papers, most of them not useful for this project. In order to rationalize the literature search, the approach of more focused research based on specific key words was applied for the fungal infection cycle with positive results and consequently it was followed also for the other issues.

The literature was included in an Endnote database collecting all citations regarding relevant topics (Appendix K). Then, the content of publications was analysed by the experts from each group involved in the project, according to their expertise, through the reading of their titles, for the first screening, and then of their abstracts; only those containing information relevant for fungi (*A. flavus* and *A. parasiticus*) or crops (maize, wheat and rice) considered in our projects and with possible presence of data important for model development were selected and included in more restricted Endnote database (Appendix L).

For the selection of papers/reports relevant for the project, some exclusion criteria were fixed in order to facilitate the development of the definitive and restricted database.

In particular, from the complete file, only relevant records were selected taking into account the following exclusion criteria:

commodities different from maize, wheat and rice,

post-harvest studies,

details not useful for practical use, e.g. the role of fungal genes in aflatoxins synthesis,

economics aspects, and

toxicity and mycotoxins in animals.

The selection of relevant papers was easy because those excluded were not related with the issues described in the report.

Full papers were collected and considered for the preparation of the reports and the Excel file.

Excel files have been prepared to collect all quantitative data available, useful for modelling *A. flavus* and *A. parasiticus* growth and AFs production, and the reference source was included. These files have been prepared taking into account each step of infection cycle of *Aspergillus* section *Flavi* relevant for modelling. In particular, five excel files have been prepared: sporulation, dispersal, germination, growth and AFs production **(**Appendices M-Q).

2.2. Modelling *Aspergillus flavus* in maize

Information on *A. flavus*-maize pathosystem was organized in a relational diagram according to the principles of the "systems analysis" (Leffelaar and Ferrari, 1989; Figure 10). In this diagram, state variables define the status of the system at a certain time and rate variables regulate the flow from a state to another. The rate variables are driven by constants or driving (external) variables; intermediate variables are sometimes considered, intended as variables computed from driving variables. Mathematical functions are associated to each rate variable. Data from literature, or from experiments specially arranged by the authors to fill the gaps, were used to elaborate the functions with the statistical package SPSS (PASW 18).

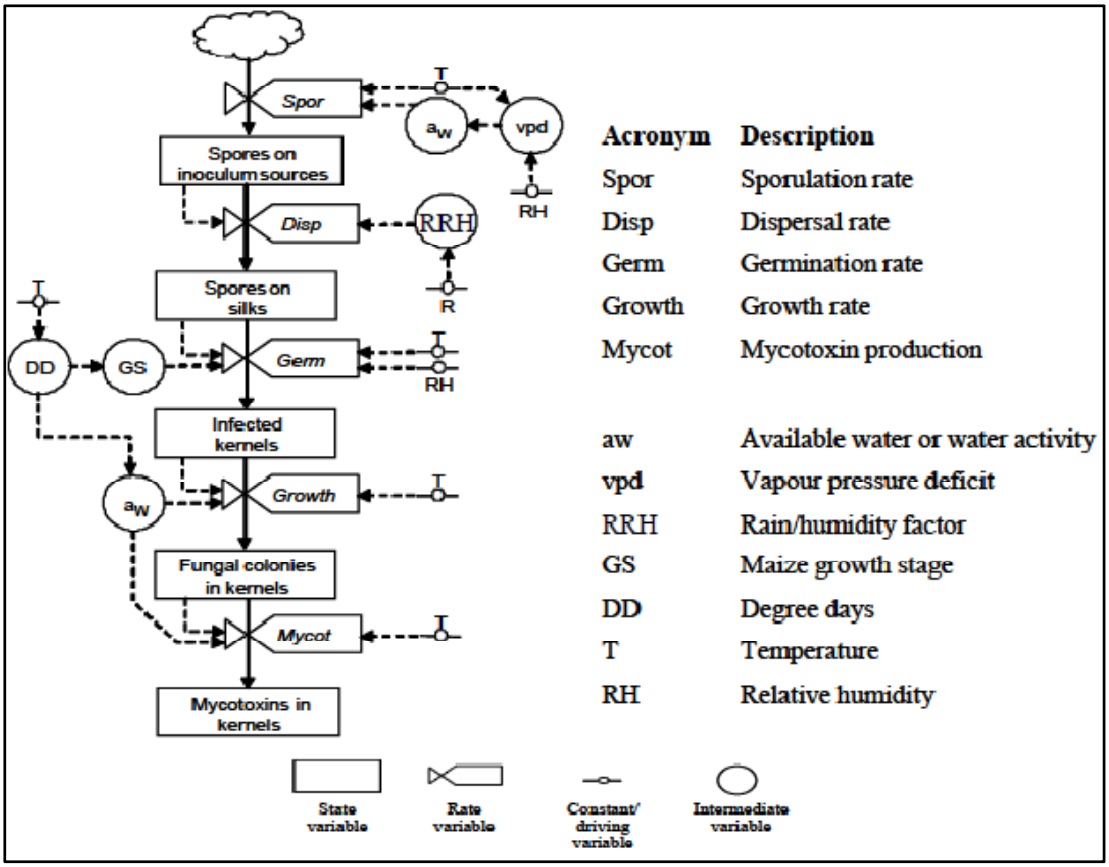

Figure 10: Relational diagram of the predictive model for *Aspergillus flavus* growth and aflatoxin production in maize

It is reasonable to consider this relational diagram appropriate also for *A. parasiticus* because these 2 fungal species are very close to each other, they have the same infection cycle and a very similar behaviour on host plants, according to the literature (Bhatnagar et al., 2006). Nevertheless, information available for *A. parasiticus* is limited and was is not possible to develop all the mathematical functions describing the rates as done for *A. flavus*.

All papers with quantitative data useful to develop mathematical functions to be included in the predictive model are summarised in this section.

2.2.1. Overwintering

Aspergillus flavus and *A. parasiticus* overwinter in soil as conidia, sclerotia and hypae, which act as primary inocula for maize infection (Horn, 2003). Sclerotia and conidia of *A. flavus* survive winter at high rate (93 % in soil). The number of propagules generated in soil varies according to the fungal strain and location, but usually it is maximum after the first growing season with maize (Wicklow et al., 1993). However, comparing maize cobs left in field or in soil as sources of *A. flavus* inoculum, it has been seen the higher importance of the former compared to the latter. The quantity of *A. flavus* inoculum in maize cobs decreases with maize cob age, but cobs can represent a long-term source of inoculum (Jaime-Garcia and Cotty, 2004). No quantitative data on *A. parasiticus* overwintering are available in literature.

Because of the lack of precise data, the inoculum is assumed to be present in any maize growing areas even though the disease is known to not occur. The outbreak of *A. flavus* epidemic and severe AFs

contamination in maize grown in northern Italy in 2003, never signalled before, supports this assumption (Piva et al., 2006).

2.2.2. Sporulation

No studies on spore production by *A. flavus* depending on different ecological conditions are available in literature; it has been demonstrated that under conditions of high temperature and low a_w, conditions which are usually associated with drought in temperate agricultural crops, *A. flavus* is very competitive and may become the dominant fungal species in the soil (Payne, 1998). Using different concentration of *A. flavus* inoculum in soil, the highest infection and contamination of maize was observed with the highest level of inoculum, indicating a direct relationship between soil population and extent of infection and AFs contamination occurring in pre-harvest (Wicklow et al., 1993). No quantitative data on *A. parasiticus* sporulation at different ecological conditions are available in literature.

A specific experiment was managed by this consortium in order to quantify *A. flavus* sporulation, both on artificial media and on maize stalk pieces, as a function of T and a_w. Sclerotia have been chosen as overwintering inoculum.

One strain of *A. flavus* (A 2062) stored in the fungal collection of the Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore, was transferred on CZ media (Czapek agar: sucrose 30 g; $NaNO_3$ 2 g; KCl 0.5 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; $FeSO_4 \cdot 7H_2O$ 0.01; K_2HPO_4 1 g; $ZnSO_4 \cdot 7H_2O$ 0.001 g; $CuSO_4 \cdot 7H_2O$ 0.005 g; agar 15 g; H_2O to 1 L) to promote sclerotia production.

After 14 days, sclerotia developed were used to artificially inoculate both Petri dishes with artificial media (CZ) and maize stalks pieces (around 3-4 cm length). Maize stalks pieces were collected in maize fields after harvest time and were autoclavated twice to avoid possible contamination from other fungal species.

Each Petri dish and each piece of stalk was inoculated putting one sclerotium of *A. flavus* on the centre of the substrate. Incubation was done at different temperatures (5-45 °C). The a_w level considered for this trial was 0.99 a_w. The stalks were incubated in boxes containing pure water on the bottom able to saturate the air present around them. Sporulation has been quantified after 20 days of incubation, with 0.25-4 days step.

Water activity between 0.50 and 0.99 were also considered in the trial with incubation T at 25 °C. The modification of a_w was done adding glycerol to artificial media (for CZ Petri dishes) or salt to pure water in boxes for stalks. The dosage of glycerol added was defined in agreement with Giorni et al. (2011). Sporulation has been checked after 8 and 20 days of incubation. All the trials have been managed in triplicate (Giorni et al., in preparation).

2.2.3. Spore dispersal

Aspergillus spore dispersal is negatively correlated with relative humidity (RH) and positively correlated to wind (W) speed (Li and Kendrick, 1995).

Airborne conidia of *A. flavus* can travel short distances, between 2-14 m, the spore concentration decreasing linearly with the distance from the source, independently from the growing season and place (Olanya et al., 1997). Data collected in the field showed that no dispersal is possible in rainy days (Abdalla, 1988; Battilani et al., unpublished).

Quantitative data on *A. parasiticus* spore dispersal are not available in the literature.

2.2.4. *Spore germination*

The minimum a_w for *A. flavus* spore germination is around 0.80 with temperature between 25 °C and 37 °C, on artificial media, after 100 days of incubation, according to Pitt and Miscamble (1995), while Ayerst (1969) observed germination at 0.78 a_w and 33 °C after 95 days of incubation.

At optimal conditions (a_w = 0.99 and T > 25 °C), *A. flavus* spore germination occurs in few hours and it is faster at 37 °C. Little difference exists in time requested for spore germination at 25 °C and 30 °C, except at low a_w where at 30 °C germination is faster (Pitt and Miscamble, 1995).

On maize plant, spore germination firstly occurs close to the pollen grains and germ tubes grow rapidly across the silk producing extensive growth and branched hyphae. On green, but not pollinated silks, only a few conidia germinate 24 hours after inoculation and they fail to establish significant mycelial growth (Marsh and Payne, 1984a). This underlines the relevance of maize growth stage for spore germination and it suggests silk browning, around 7-10 days after BBCH 65, as the starting point for ear infection. No study defines the time ears remain susceptible to *A. flavus* infection.

In another study, spore germination was observed after 4 hours at optimal conditions (a_w > 0.95 and T > 25 °C), but time increased largely with a decrease in a_w level and temperature. In particular, at 0.85 a_w, 30, 40 and 50 hours were necessary for germination at 37, 30 and 25 °C, respectively (Marín et al., 1998). At optimal a_w conditions (> 0.95 a_w) and at temperatures in the range of 15-45 °C, spore germination is always possible. With decreasing a_w, the percentage of germinated spores decreases and ceases at 0.80 a_w, independently of temperature (Marín et al., 1998; Schmidt-Heydt et al., 2010).

No quantitative data on *A. parasiticus* spore germination at different ecological conditions were available in the literature.

2.2.5. *Fungal growth*

Optimal conditions for *A. flavus* growth are 33 °C and a_w above 0.98, with 12 and 43 °C and 0.78 being the limit for temperatures and a_w respectively in *in vitro* trials (Ayerst, 1969).

Data obtained by different authors are consistent; at temperatures above 22 °C, growth occurs at all a_w tested and it is positively and linearly correlated to a_w from 0.99 to 0.85. At 15 °C, *A. flavus* grows only with $a_w \geq 0.95$, not at 0.90 a_w or 0.80 a_w (Holmquist et al., 1983).

In the *in vitro* study managed by Pitt and Miscamble (1995), the optimum a_w for growth was in the range 0.96-0.98 a_w at 25 °C, 0.985 at 30 °C and 0.96 at 37 °C. The optimal ecological conditions for *A. flavus* growth at 30 °C and 0.99 a_w were confirmed also by Marín et al. (1998) and Samapundo et al. (2007). Generally, a reduction in a_w level respect to the optimal 0.99 a_w, determines a reduced fungal growth. In particular, decreasing available water to 0.95, 0.93 and 0.90 a_w cause 47 %, 74 % and 95 % reduction in growth rate, respectively (Giorni et al., 2011).

Data on growth at different ecological conditions are available also for *A. parasiticus* (Holmquist et al., 1983; Pitt and Miscamble, 1995). Elaboration of data showed that the behaviour of *A. parasiticus* is very similar to *A. flavus* regarding growth both at different temperature and a_w. For this reason, the equations drawn for *A. flavus* can be considered useful also for *A. parasiticus*.

2.2.6. *Aflatoxins production*

Few studies considered AFB_1 production by *A. flavus* at different conditions of a_w and T; no data are available in literature on the production of the other AFs in different ecological conditions. Most strains of *A. flavus* produced the highest quantities of AFB_1 at 25 °C, while at 15 °C and 30 °C the number of aflatoxigenic strains decreased as did the amount of AFB_1 produced (Giorni et al., 2007; Schmidt-Heydt et al., 2008).

Only two papers have been published on AFB_1 production by *A. parasiticus* (Marin et al., 2001; Schmidt-Heydt et al., 2010). The behaviour of this fungus regarding mycotoxin production seems to be slightly different respect to *A. flavus*. In particular, the optimal temperature for AFB_1 production resulted to be higher compared to *A. flavus* (37 °C *versus* 30 °C) and the effect of low a_w seems less influent. However, at the moment, data on *A. parasiticus* are not sufficient to be used for modelling.

2.2.7. Maize growth stages

Maize growth stages are crucial for the model because spore germination on ears starts after BBCH 65 so as plant susceptibility; that is also the plant growth stage when infection can take place. Besides, a_w is a crucial parameter in all the steps of *A. flavus* infection cycle and it decreasing during ripening.

A maize phenology model has been linked with the *A. flavus* model in order to jointly predict potential infection and plant susceptibility.

2.2.8. Model development and sensitivity analysis

Regression analysis was applied to all quantitative data available in order to define all the rates described in the relational diagram: SPOR, DISP, GERM, INF, INV and AF (see 2.2 for details). All the rates were measured in a 0-1 scale, with 1 intended as the maximum possible rate (optimal conditions for that step of infection cycle); when the rate assumes the value 0 it means that the step is not possible due to the environmental conditions.

Sensitivity analysis is a useful step to identify variables that are likely to contribute to model errors. The analyses of response linearity to the range of input values (Rastetter et al., 1992) and sensitivity of mean response to the variance of inputs (Addiscott, 1993) provide an idea of the potential for bias due to heterogeneity of data input. Sensitivity of a certain model output to a given parameter can be defined as the rate of output change resulting from a variation of data input while keeping all the other parameters constant (Wöhling, 2005).

A sensitivity analysis of the AF predictive model has been done in order to understand the relevance of changing the meteorological parameters T and RH on the final output.

Present scenario in year 2050 and wheat crop have been considered in the whole European grid. Twelve simulations have been run, six increasing/decreasing T (2 °C, 4 °C and 6 °C) and six increasing/decreasing RH (10 %, 20 % and 30 %).

2.3. Crop growth modelling

2.3.1. Modelling wheat and maize phenology

For each of three IPCC scenarios for the year 2100 (present, + 2 °C, + 5 °C), shifts in crop development stages were simulated. The approach taken for each of the two crops of wheat and maize was based on application of the Crop Growth Monitoring System (CGMS). CGMS has been developed by the MARS project (Monitoring Agriculture with Remote Sensing) with the aim to provide the European Commission (DG Agriculture) with objective, timely and quantitative yield forecasts at regional and national scale. CGMS monitors crops development in Europe, driven by meteorological conditions and modified by soil characteristics and crop parameters. CGMS integrates crop growth modelling, a relational database (ORACLE) and GIS (ARC/INFO) with system analytical part for yield forecasting. There are databases on soil, weather, crop, and yield statistics that cover the entire Europe. The system-analytical part consists of three modules, one of which is the crop growth module. This growth module consists of the dynamic simulation model WOFOST in which crop growth is calculated and crop indicators are generated. In CGMS, WOFOST is run on a daily bases for each so-called 'simulation unit', i.e. a unique combination of weather, soil, and crop (mapping) units. In the statistical module, crop indicators (total above ground dry weight and dry weight storage organs) calculated with WOFOST are related to historical yield statistics through regression analysis in combination with a

time-trend, for at least 15 years of simulated and historical data. The resulting regression equations per crop per region are used to make actual yield forecast.

For the aims of the current study, the crop regression equations for maize and wheat were used to estimate the shift in crop development (including, amongst others, flowering date and harvest date), needed for fungal and mycotoxin modelling. Underlying databases with information on cultivation of maize and wheat as well as temperature requirements for crop development stages, both per grid (50 x 50 km) in Europe, were used. Climate data was available for each of 2254 weather stations, identified with a positional latitude and longitude. The CGMS/JRC grid is 50 x 50 km (polygon) and mapping has been done by calculating the nearest weather location for each grid using closest Euclidean distance. This process was done by standard ESRI GIS software.

Table 8: Degree day requested to reach flowering (Tsum1) and harvest (Tsum2) indifferent maize and wheat varieties according to JRC data set.

Maize variety	Tsum 1	Tsum 2	Wheat variety	Tsum 1	Tsum 2
1	750	700	1	600	970
2	950	775	2	800	750
3	950	875	3	800	970
4	1000	1000	4	800	1100
5	1050	1050	5	1000	970
6	950	950	6	1100	750
7	900	1100	7	1200	970
			8	1200	1100
			9	1350	970

The database with temperature requirements included the DD needed – relative to the starting point of simulation, to reach a particular crop stage. The simulation starting date is the crop emergence date, which was set at 1 January for wheat, and at the sowing date plus a fixed value (110) for maize. A fixed value, per grid, of temperature sum requirements is then used to estimate flowering date and harvest date relative to emergence date. Temperature sum (TSUM) requirements for reaching flowering (TSUM1) and harvest (TSUM2), relative to emergence date, of both crops are available in the database for each grid. Nine and seven varieties were considered for wheat and maize respectively and they were placed in different geographic areas (Table 8). The maximum length of growing season is fixed in 200 days from emergence and 300 days from 1 January respectively for maize and wheat.

2.3.2. Modelling wheat and maize scenarios

For each of three IPCC scenarios (present, +2 °C, +5 °C), shifts in crop development stages were simulated. This was done by 100 runs (100 years generated meteorological data) for each relevant grid in Europe. Using the crop data provided by CGMS, in total 2085 grids were available for wheat and 1573 for maize. TSUM requirements (from the database) and climatic forecasts were used as input into the crop growth equations. Output includes the dates for crop development stages (flowering date, harvest date) for both wheat and maize, per grid size.

The results were statistically analysed for the shifts in flowering and harvest dates, for both of wheat and maize, as well as their differences (indicating the length of the season) due to climate change. To this end, the 50^{th} and 90^{th} percentiles of the flowering date, harvest dates, and difference between flowering and harvest dates, as based on the 100 runs per grid, were calculated. Then histograms of these percentiles were made using all grids both for wheat and maize. From the 100 simulations run

(100 simulated years) six runs were selected (covering the entire Europe), based on the meteorological data analysis. The less favourable conditions for AF development included a wet and cold year, and the best conditions represented a warm and dry year. From the selected runs from each of the three climate scenarios, flowering date and harvest date for wheat and maize were calculated per grid. In total, six different climate change scenarios were considered for crop phenology prediction (Table 9).

Table 9: Number of scenarios for crop growth in relation to climate change

Scenario	Climatic scenario	Crop model	FD and HD	No. grids	Years
1	Present	Wheat JRC/MARS	TSUM1, TSUM2	2085	100
2	+2 °C	Wheat JRC/MARS	TSUM1, TSUM2	2085	100
3	+5 °C	Wheat JRC/MARS	TSUM1, TSUM2	2085	100
4	Present	Maize JRC/MARS	TSUM1, TSUM2	1573	100
5	+2 °C	Maize JRC/MARS	TSUM1, TSUM2	1573	100
6	+5 °C	Maize JRC/MARS	TSUM1, TSUM2	1573	100

(FD): Flowering date
(HD): Harvest date

2.3.3. Modelling rice phenology

Crop growth modelling of rice is based on application of the Water Accounting Rice Model (WARM) that has been the result of an unofficial cooperation among researchers working at the JRC (IPSC, Agrifish Unit – MARS STAT Section), at the Department of Crop Science of the University of Milan, at the Istituto Sperimentale per le Colture Industriali (Consiglio per la Ricerca e la Sperimentazione in Agricoltura; CRA – ISCI) and at the Institute for Electromagnetic Sensing of the Environment (IREA-CNR) of Milan. This interaction had the aim of developing a simulation model for flooded rice able to manage all the main aspects influencing crop production (e.g. crop behaviour, pests, weeds). WARM is an original model with several innovative features useful for reproducing the peculiar conditions of mid-latitudes paddy fields (e.g. floodwater effect on vertical thermal profile).

WARM is used within the crop yield forecasting system of the European Commission and has been firstly evaluated for the simulation of rice growth under flooded and unflooded conditions in China and Italy and therefore validated at five rice districts of different countries (i.e. France, Greece, Italy, Portugal, and Spain). The WARM model simulates crop growth and development, floodwater effect on the vertical thermal profile, blast disease, cold-shock induced spikelet sterility during the pre-flowering period and hydrological peculiarities of paddy soils. The most relevant model parameters were identified through sensitivity analyses carried out using the Sobol' method and then calibrated using the simplex algorithm.

WARM has shown robustness and accuracy, combined with the low requirements in terms of inputs. The implementation of modules for reproducing biophysical processes, strongly influencing the year-to-year yield variation, makes the model suitable for forecasting rice yields on regional, national and international scales.

Temperature (strongly influenced by floodwater in paddy fields) is one of the most important driving variables for simulating crop growth and development, and the interaction between crops and pathogens. WARM pre-processes maximum and minimum daily temperatures using the micrometeorological model TRIS (Confalonieri et al., 2005) to get hourly water and air temperatures (18 layers of 0.1m each starting from soil–water interface) as influenced by floodwater. Temperatures, generated at the meristematic apex height, are used to simulate crop development and spikelet sterility, whereas average canopy temperature is used to introduce thermal limitations to photosynthesis and leaves aging. For crop development, the thermal time accumulated between a base temperature and a cut-off temperature is computed. The accumulated thermal time can be optionally corrected with a

factor accounting for photoperiod. Considering the processes related to crop growth, net photosynthesis is simulated using radiation use efficiency (RUE) approach, converting the solar radiation intercepted by the canopy into aboveground biomass. WARM takes into account relevant processes influencing the rice growth and yield, while keeping the number of parameters small.

The simulation starting date is the sowing date. Fixed values of temperature requirements and growing degrees days (DD) are used to estimate emergence, flowering and maturity dates relative to sowing date. To determine daily DD accumulation, calculate the average daily temperature (high + low)/2 and subtract the base temperature which is 12 °C for rice. The development parameters for the different growth stages are summarised below in Table 10.

Table 10: Values of environmental parameters useful for the prediction of rice growth stage

Development parameters for the different growth stages		
Pre-emergence	Base T[a]	12
	Maximum T	42
Post-emergence	Base T	12
	Maximum T	42
	DD[b] to emergence	100
	DDs emergence - flowering	800
	DDs flowering – maturity	430
	Growth parameters for biomass accumulation	
Ripening	Base T for biomass accumulation (°C):	12
	Optimum T for biomass accumulation (°C):	28
	Maximum T for biomass accumulation (°C):	35
Growth parameters for net photosynthesis		
	Radiation use efficiency (RUE)[c] (MJ)	2.3

([a]): T measured in °C
([b]): DD measured in °C-days
([c]): RUE = grams of biomass (above ground dry matter)/Mega Joule of intercepted radiation (photosinthetically active radiation)

2.4. Climate change scenario data generation

2.4.1. Model implementation

This session describes the implementation of a weather generator to be used in climate impact assessments of agricultural management. The generator produces internally consistent series of meteorological variables including T_{min}, T_{max}, rainfall, radiation and humidity. The resolution of the system is of daily time, applying a GCM (Arnell, 2004) statistical downscaling procedures based on the LARS –WG 5.0 version (Semenov and Porter, 1995; Semenov and Stratonovitch, 2010) that includes climate scenarios based on the fourteen GCMs which have been used in the latest IPCC 4th Assessment Report (2007).

Observed daily data (T_{min}, T_{max}, rainfall, radiation, RH) for the period 1975-2005, spaced 50 x 50 km over the EU domain, have been used for the local calibration of the stochastic weather generator. After calibration, 100 years of synthetic daily weather data have been produced for each grid point to represent the baseline period over the domain. These data are reported as years between 2000 and 2100; nevertheless, the prediction is not intended as related to the specific year, but only a time series of 100 simulated years. In other words, i.e. 2050 is not the prediction of year 2050, but only the 50th prediction.

The results of Hadley Centre Coupled Model, version 3 (HadCM3) for A2 and B2 scenario storyline (Carter, 2001; Fisher et al., 2007; Morita, 2001; Nakicenovic, 2000) have been used to derive the forcing factors for the downscaling procedure. Two global scenarios have been chosen, belonging to the A2 and B2 scenario storylines, because this choice partly covers the range of uncertainty associated with the driving forces of global emissions: demographic change, economic development, technological change.

This allowed to reproduce, on a regional scale, the future climate corresponding to 2 global scenarios belonging to +2 °C and +5 °C (above preindustrial levels) scenario storylines. A downscaling procedure was set up in order to reproduce, on a scale suitable for impact assessment in agriculture (i.e. 50 × 50 km), the future climate at an average global warming of +2 °C and +5 °C. This procedure

was based on the use of the LARS weather generator (LARS – WG) that allowed including changes in mean climate as well as in climate variability as derived from GCM in future climate simulations. These has been computed for each GCM grid point over the domain, as monthly average differences of T_{min}, T_{max}, rainfall, radiation and relative humidity between the future and the reference period (1975-2005). Finally, forcing factors calculated for each GCM grid have been applied in the downscaling procedure to perturb the relevant climatology of the observed dataset generating stochastically 100 years of daily data for each 50 x 50 km grid point. The effectiveness of LARS WG in reproducing present climate was preliminary tested carrying out a statistical comparison of the weather generator outputs with the observed data over a sample of 100 grid points randomly selected across the domain.

Generated and observed mean monthly data were compared to provide a general overview of LARS WG performances on the mean local climate generation. Additionally, the quarterly probability distributions for length of wet and dry series, frost (days with minimum temperature less than 0), and warm series (days with maximum temperature greater than 30) and the monthly probability distributions for precipitation were compared to test LARS WG reliability in simulating climate variability. The probability distributions for the generated and observed data were compared using the chi-square (χ^2) goodness of fit test. The means were compared using the t-test. In each case a p-value was calculated, which is used to accept or reject the hypotheses that the two sets of data belong to the same distribution.

An example of the grid applied visible on part of EU is shown in Figure 11; all European countries have been included in our procedure. These data were generated in a geographical information system. At this point (Semenov and Stratonovitch, 2010) these data, generated in a geographical information system at 50 x 50 km grid point resolution, have been interpolated combining multiple regression with inverse distance-weighted interpolation taking account of geographic and orographic factors, to obtain information about places of interest.

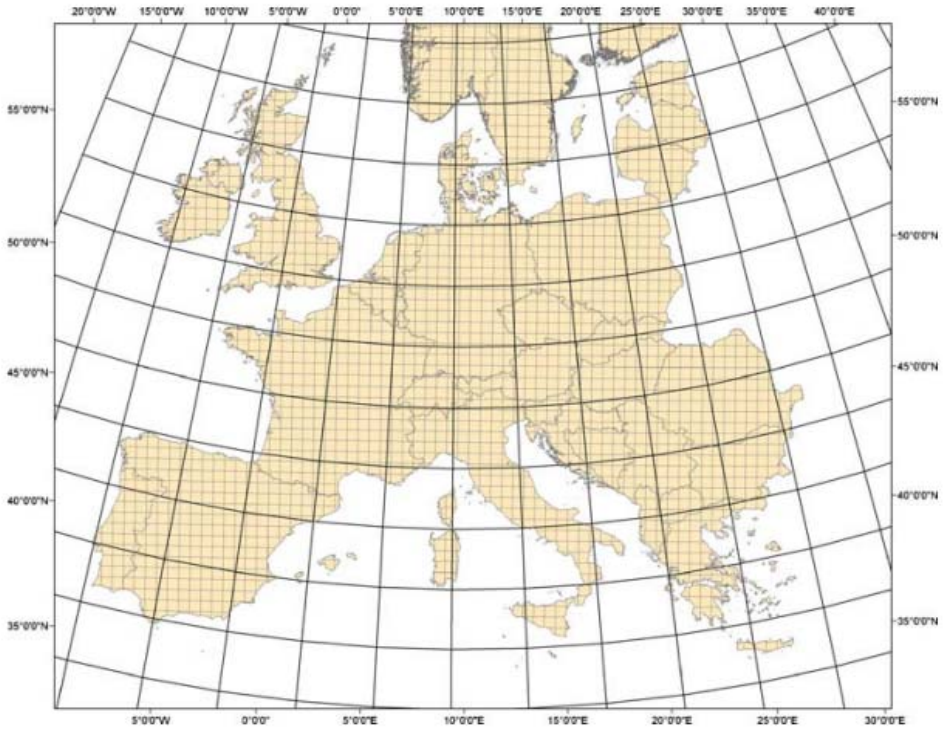

Figure 11: Example of the grid applied visible on part of EU. Hadley Centre Coupled Model, version 3 (HadCM3 GCM) grid (bold line, 3.75° Lon×2.5° Lat) overlaid to the grid of the observed dataset (thin line, 0.5°×0.5°). HadCM3 climate perturbing factors (i.e. the monthly average differences of Tmin, Tmax, R and Radiation between the future and the baseline period [1975–2005]) were statistically downscaled over the relevant grid points of the observed dataset

Similarity in the nature of the distributions of the weather variables for nearby sites is expected since these sites will normally be subject to the same basic type of weather on each day. However, systematic differences can occur, particularly if the sites are at significantly different elevations, with precipitation tending to increase and temperature tending to decrease with elevation. The interpolation procedure devised consists of an initial local interpolation in which the weighted average of the weather generator parameters for three neighbouring sites from the database are calculated. The precipitation and temperature distributions of the target site were adjusted for the site elevation. Precipitation-elevation and temperature-elevation relationships were obtained from global interpolation of monthly average precipitation and temperature by thin plate spline (Hutchinson, 1995) functions using elevation as an independent variable in addition to the geographical coordinates. The parameters for precipitation and temperature at the target site were then adjusted based on the mean values predicted by the spline functions.

2.4.2. Weather data generation

Scenarios of weather data have been produced using state-of-the-art technology as described in 2.4.1 Model Implementation. These data represent the data input for crop phenology and *A. flavus* models. Therefore, a complete dataset for each grid point and for each scenario (present, +2 °C, +5 °C) has been provided, including this additional parameter. For each grid point the daily mean temperature was calculated as the average of the given minimum and maximum daily temperature, implementing a command string within an automatic code developed in Matlab for data quality check, the files storage and for the GIS mapping.

Once the weather data have been produced for each scenario, the entire database has been organized in a main folder (EFSA meteo data) and in three subfolders (Present Scenario, Scenario + 2 °C and Scenario + 5 °C). For each subfolder, belonging weather data have been stored in ASCII files, one for each grid point, with a file name that identifies the grid point and the scenario:

(i) w_ID_BaseWG, for present scenario

(ii) w2_ID_anomalyWG, for scenario + 2 °C

(iii) w5_ID_anomalyWG, for scenario + 5 °C

Each file has 8 columns: year, Julian day, minimum temperature (°C), maximum temperature (°C), rain (mm), global radiation (MJ/m^2), relative humidity (%) and average temperature (°C).

A list of all grid points with their ID, geographical coordinates (Lat/Long decimal degree, WGS84) and altitude (above sea level) has been prepared.

2.4.3. Meteorological data analysis

A statistical meteorological data analysis was performed in order to choose years to be used for crop phenology and *A. flavus* model simulation. This has been done because it is practically impossible to show all data and produce maps for 100 simulated year data.

The analysis was performed on the entire domain (2254 grid points) and for all the three scenarios (present, + 2 °C, + 5 °C).

The analysis was focus on three different short-term periods including the flowering stage of wheat maize and rice, being crucial for crop susceptibility to *A. flavus* infection (see 1.1.1.1, 1.1.1.2 and 1.1.1.3). The three relevant periods are described below:

Wheat: 1 - 31 May

Maize: 15 June – 15 July

Rice: 1 – 31 August

The analysis reported refers to the + 2 °C scenario and the period 11-100 years, considering the temperature and precipitation dataset.

The + 2 °C scenario has been chosen for the meteorological data analysis because a comparison of true climate data for the years 2000-2009 and the first 10 years of the simulated data, it was found that current conditions at European scale are close to those predicted with the + 2 °C scenario.

For each crop and each relative short-term period, temperature and rain data were analyzed as follows:

(i) Scatter-plot, in order to detect the distribution shape and the years characterized by “extreme” and “average” values. A set of years representative of the entire inter-annual variability were chosen

(ii) Probability plot, in order to highlight the difference between the selected years

(iii) Maps for temperature and precipitation, for each year selected, were drawn.

(iv) Macroareas were identified taking into account both climate characteristics and latitude to describe with a better geographic detail the variability of temperature and rain (Table 11):

Table 11: List of countries per macroarea

Macroareas names	Countries per macroarea
BALKGREE	Balkans, Bulgaria, Greece, Slovenia, Cyprus
BALTIC	Estonia, Latvia, Lithuania
EEUROPA	Slovakia, Hungary, Romania, Poland
ENG	Ireland and United Kingdom
IBERIA	Spain and Portugal
ITALIA	Italia, Malta
MIDDLEEU	France, Switzerland, Austria, Belgium, Netherlands, Germany, Czech Rep, Denmark
SCAND	Norway, Sweden, Finland

2.5. Dataflow

In order to model climatic scenarios and predict their effect on occurrence of AFs in a spatially explicit way, consistent data handling is important to be able to link models on crops, climate, fungi development and toxin production.

A stepwise dataflow and crucial model links follows and a graphical depiction is given in Figure 12:

Step 1. Meteorological data generation.

Simulated years with meteorological data on a daily base have been generated based on each climate scenario considered (actual, + 2 °C, + 5 °C). This is done by weather generators (see Section 1.5.1.2 for details) that produce seasonal time series (100 series) for each GIS grid cell on a European scale. These time series are the input for both the crop phenology models and the fungal infection and AFs model. Statistical data analysis has been managed to select specific years for mapping.

Step 2. Crop phenology prediction.

Using temperature requirements and crop phenology from the MARS JRC database and temperature series from Step 1, flowering dates and harvest dates have been calculated for each GIS grid cell for each year for maize, wheat and rice. These data are the base for statistical analysis and comments on the variation of crops phenology in different climate change scenarios, mapping flowering date and comments on the window of crops susceptibility to *A. flavus.*

Step 3. *A. flavus* predictive model development and running.

The *A. flavus* predictive model has been developed. Fungal development and AFs production were modelled using grid/year specific meteorological data time series generated in Step 1 that are framed in time windows around flowering dates and up to harvest dates generated in Step 2. The output generated in Step 3 consists of yearly maps with the risk of AFs contamination in Europe in different climate change scenarios in the selected crops (Figure 12).

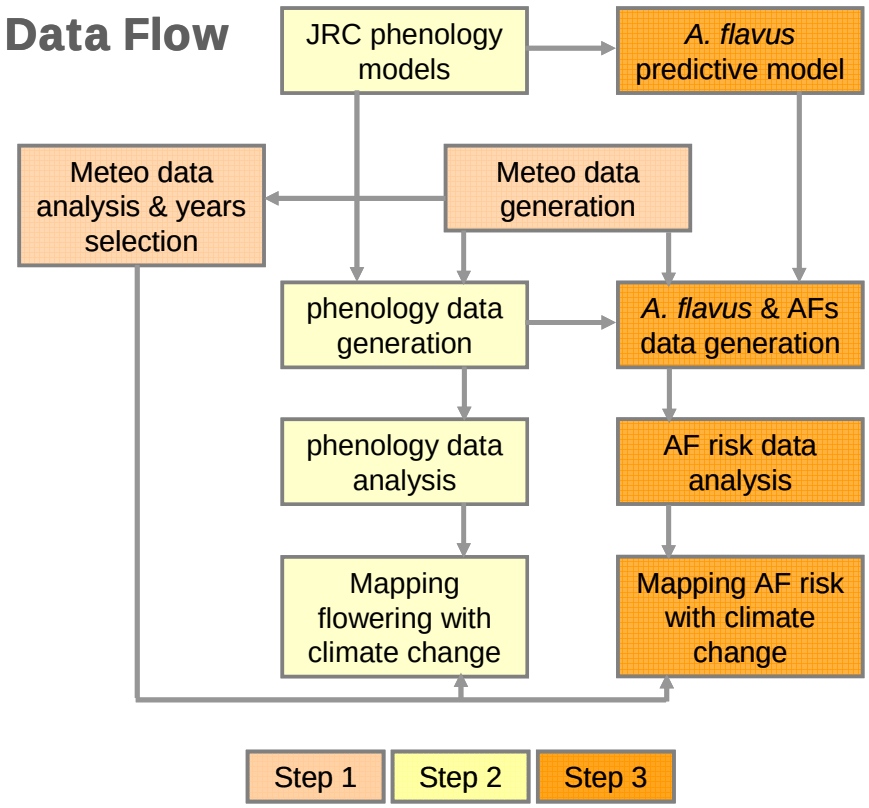

Figure 12: Summary of data flow followed in this project. Three different steps were considered, all strictly related to each other

3. RESULTS

3.1. Modelling *Aspergillus flavus* in maize

3.1.1. Sporulation

No data were found in the literature regarding *A. flavus* sporulation, but the trial organised in this study gave a suitable input. Sporulation was observed between 10 °C and 40 °, and in the whole a_w interval considered, even though it was limited up to 0.7 a_w (Giorni et al., in preparation).

The dynamic of spore production (SPO) in different T and a_w regimes were fitted by Bete[10] and logistic equations, respectively, as follows:

$$SPO(T) = \left(a * \left(T_{eq}\right)^b * \left(1 - T_{eq}\right)\right)^c$$

where Teq is the equivalent of temperature, computed as follows:

$$T_{eq} = \frac{\left(T - T_{\min}\right)}{\left(T_{\max} - T_{mim}\right)}$$

where T is the daily mean T, T_{min} and T_{max} are the cardinal of temperature with T_{min} = 5 °C and T_{max} = 45 °C

$$SPO(a_w) = \frac{a}{\left(1 + \exp\left(b + c * a_w\right)\right)}$$

Based on the obtained results, both T and a_w were found to be relevant for *A. flavus* sporulation.

No published data were available regarding a_w dynamic in stalk residues in soil, the main source of inoculum, during maize flowering, the period of ear susceptibility. Some data were produced by Battilani et al. (unpublished) and summarized in Figure 13. Water activity measured in maize stalks varied between 0.4-0.85 a_w. The lowest value was recorded after almost 1 month with no rain (Figure 13).

Sporulation was observed, in the *in vitro* trial, in the whole a_w interval studied (from 0.5 to 0.99 a_w), even if it decreased significantly below 0.7 a_w. It was reasonable to consider that a_w is commonly not a limiting factor for sporulation and that SPO could be computed with the Bete equation as a function of T.

[10] This function was developed by Analytis (1977) to describe mycelium growth *in vitro* in relation to temperature. It is simple and fitted well for 10 different fungi; it is commonly used to describe T dependent fungal growth

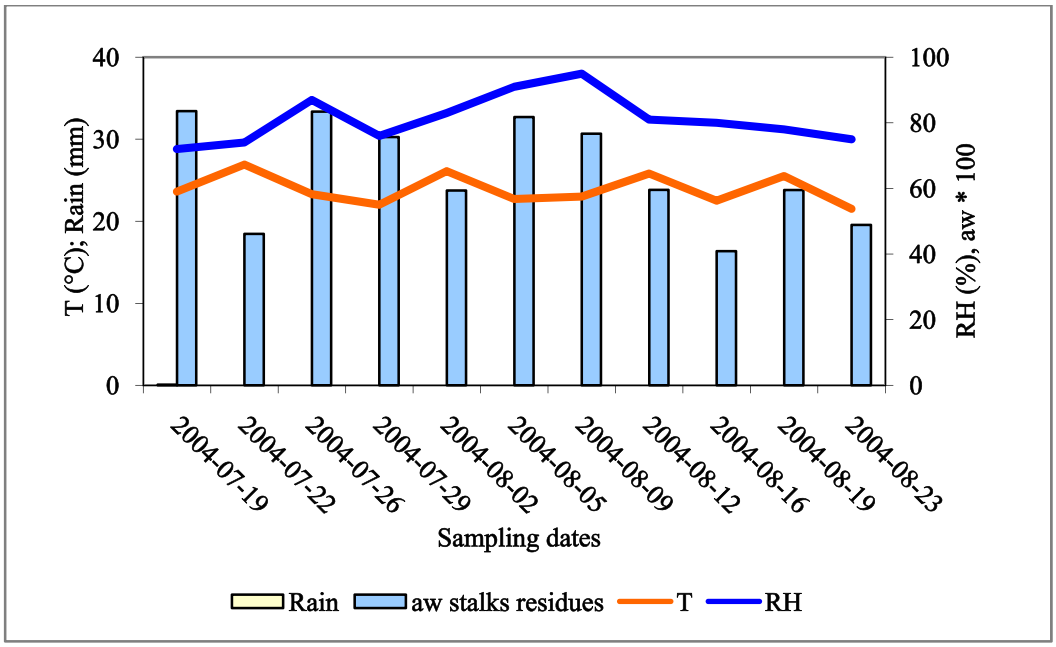

Figure 13: Dynamic of aw in stalk residues during maize growth stages BBCH 65-83, between 19 July and 23 August 2004, in north Italy (T: mean daily air temperature, RH: relative humidity)

3.1.2. *Spore dispersal*

Data collected in the field showed that no dispersal is possible in rainy days (Abdalla, 1988; Battilani et al., unpublished) (Figure 14); no or very few airborne spores are present with RH > 75 % (Battilani et al., unpublished data). For this reason, dispersal is assumed to be constant and possible only in no-rain days with RH < 75 %.

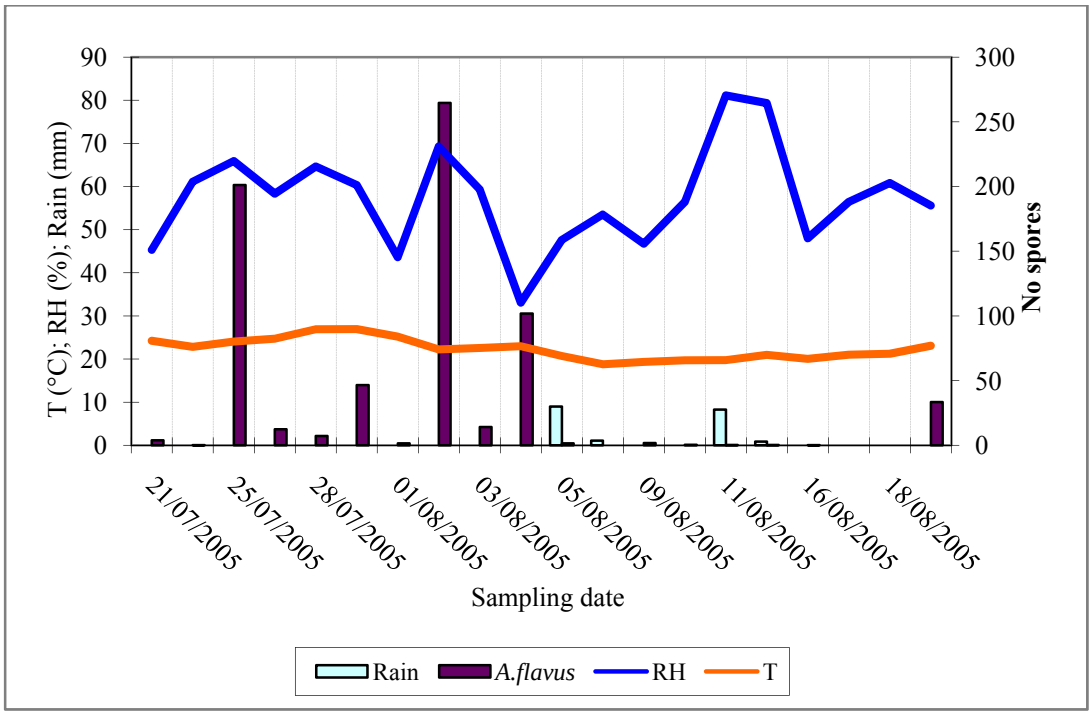

Figure 14: Dynamic of *A. flavus* spore dispersal during maize growth stages BBCH 65-83, between 21 July and 19 August 2005, in north Italy (T: mean daily temperature, RH: relative humidity)

3.1.3. *Spores germination*

Spores can germinate between 12.5–45 °C and the a_w threshold was defined at 0.84 (Ayerst, 1969; Marín et al., 1998). The a_w threshold for germination, in different T regimes, was defined as follows:

$a_w = a*T^2 + b*T + c$

Germination (GERM) was estimated using data published by Marín et al. (1998) and Ayerst (1969). The length of the lag phase for germination was computed as a function of T as follows:

$$Incubation(T) = \frac{IncubationMin}{f(T)}$$

Where:

$$f(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right) * \left(\frac{T_{\max} - T}{T_{\max} - T_{opt}}\right)^{\frac{T_{\max} - T_{opt}}{T_{opt} - T_{\min}}}$$

The GERM rate was calculated using a Bete equation as follows:

$$GERM = \left(a * \left(T_{eq}\right)^{b} * \left(1 - T_{eq}\right)\right)^{c}$$

where T_{eq} was computed with T_{min} = 10 °C and T_{max} = 47 °C.

3.1.4. *Fungal growth*

Fungal growth depends on T and a_w. All the publications reporting quantitative data were used to calculate the growth rate (GROWTH) (Ayerst, 1969; Giorni et al., 2011; Holmquist et al., 1983; Marín et al., 1998; Pitt and Miscamble, 1995). They were fitted by a Bete equation and a logistic equation, respectively for T and a_w, as follows:

$$GROWTH \quad (T) = \left(a * \left(T_{eq}\right)^{b} * \left(1 - T_{eq}\right)\right)^{c}$$

where T_{eq} was computed with T_{min} = 5 °C and T_{max} = 48 °C.

$$GROWTH\,(a_w) = \frac{a}{\left(1 + \exp\left(b + c * a_w\right)\right)}$$

where a_w is the average value measured in kernels.

3.1.5. *Aflatoxins production*

Data from the available literature were used to elaborate AFB_1 production rate (MYCOT) depending on T and a_w (Giorni et al., 2011; Schmidt-Heydt et al., 2008). The effects of T were described using a Bete equation where T_{eq} was computed with T_{min} = 5 °C and T_{max} = 42 °C. The role of a_w was described with a polynomial equation using the data produced by Giorni et al. (2011).

Based on the data collected in the field in northern Italy, AFB_1 represented 90 % of the total of AFs detected in maize (Battilani et al., 2008a, b, c). However, data on the remaining 10% AFs (i.e. AFB_2, AFG_1 and AFG_2) were not available from ecological trials. Therefore, the prediction considered AFB_1 only, as the dominant aflatoxin type in contaminated products.

3.1.6. *Dynamic of water activity in maize kernels*

Available crop growth models, (see section 1.3) do not include a_w, a parameter that is crucial for correct predictions of *A. flavus* behaviour. This aspect has been considered by Battilani et al. (2011). The dynamic of a_w in 10 maize hybrids has been described as a function of DD using a monomolecular[11] equation as follows:

$$a_w = 1\text{-}\exp(\text{-}a\text{*}\exp^{(\text{-}b\text{*DD}/100)})$$

where *a* and *b* are the equation parameters. Data included in Battilani et al. (2011) were adapted and the results are summarised in Figure 15.

[11] The monomolecular equation is a simple function based on exponentials, also named saturating exponential growth function. This function has two parameters. the initial value *a* and the growth rate *b* Seber GAF and Wild CJ, 2003. Nonlinear regression. Editor. J. Wiley & Sons, Hobeken, New Jersey,

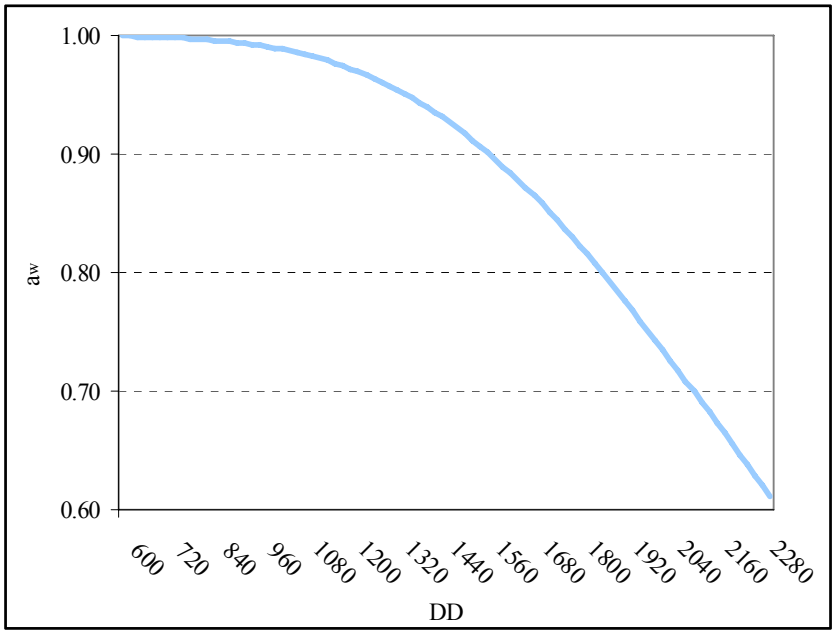

Figure 15: Monomolecular equations describe the dynamic of water activity (aw) in maize kernels defining the rate of AFB1 production as a function of aw, adapted from Battilani et al. (2011)

This function was able to fit well the dynamic of a_w in 9 of the 10 hybrids considered in relation to DD. One hybrid showed a slower a_w decrease; this is surely important, but due to the aim of this project, the level of precision obtained with equation 2 was considered reasonable and it was chosen for modelling AF risk. The studied hybrids belong to different FAO classes, from 500 to 700, and the FAO classes did not influence the equation parameters; therefore, it confirmed the broad value of the function.

3.2. Mapping of meteorological scenarios

The whole database of 100 simulated years (Appendix R) was stored by EFSA. To access the data, special requests need to be sent to the emerging risks unit of EFSA.

3.2.1. Data selection for wheat

Results from the statistical data analysis are shown in Figures 16, 17, 18 and Tables 12, 13 and 14.

The scatter plot (Figure 16) of temperature and rain data from the month of May, intended as the flowering period for wheat, highlighted the years 2016, 2020, 2021, 2072, and 2082 as extreme years, with climatic conditions described in Table 12.

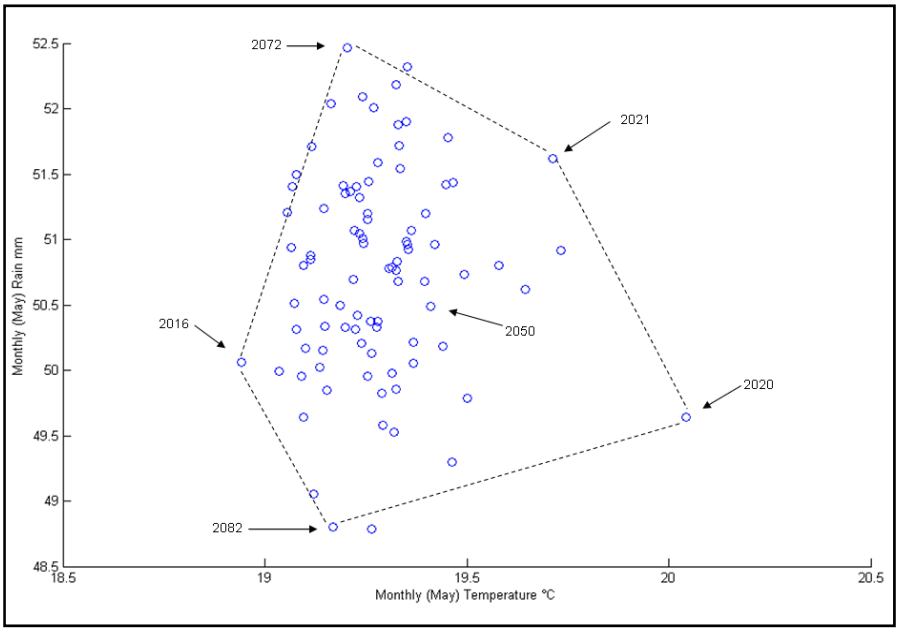

Figure 16: Scatter plot of meteorological data for the years 2011-2100: temperature and rain for the period 1- 31 May

Basic statistics of temperature and rain data (Table 12) showed that predicted rain ranged between 48.8 mm and 52.5 mm with a mean standard deviation of 38.17 mm. The mean temperature range was between 18.9 °C and 20.0 °C, a not negligible variation in May, with a mean standard deviation of 4.14 °C. Simulations for the years 2016 and 2020 were expected to show extreme values being respectively cold/wet and warm/dry years.

Table 12: Mean values for temperature and rain between 1- 31 May, for the whole set of years and for the selected years (average and five extreme years)

			Selected years					
		Set of 100 years	2016	2020	2021	2050	2072	2082
Rain (mm)	Mean	50.7	50.1	49.6	51.6	50.5	52.5	48.8
	Std	38.17	37.70	37.42	39.64	38.29	39.41	36.34
	SE	0.80	0.79	0.79	0.84	0.81	0.83	0.77
Temperature (°C)	Mean	19.3	18.9	20.0	19.7	19.4	19.2	19.2
	Std	4.14	4.16	4.07	4.29	4.10	4.12	4.11
	SE	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Year classification			**Coldest** Rainfall below average	**Warmest** Dry	Warm and wet	**Average**	**Wettest** Temp. below average	**Driest** Temp. below average

(Std): standard deviations
(SE): standard error

Considering the limited range of variation and the distribution of temperature and rain, the six selected years resumed the variability of weather conditions, including the extreme conditions, the average condition and years with different distribution of temperature and rain. A probability plot (Figures 17a, b) was drawn to compare the probability of rain and temperature in May in the six selected years. The graph highlighted the limited range of variation, as shown by the analysis performed previously with the scatter plot.

When considering the macroareas, large differences were found in May in rainfall with SCAND and MIDDLEEU as the driest and the wettest macroareas, respectively (Table 13). The extreme years were not always the same in all the considered macroareas. Less variability was observed in T_{max}, with 2020 and 2016 almost always the warmest and coldest, respectively (Table 14).

Therefore, the selected years seemed appropriate to show the variability expected within the next 100 years in rain and temperature. This variability was mapped and shown in Figure 18.

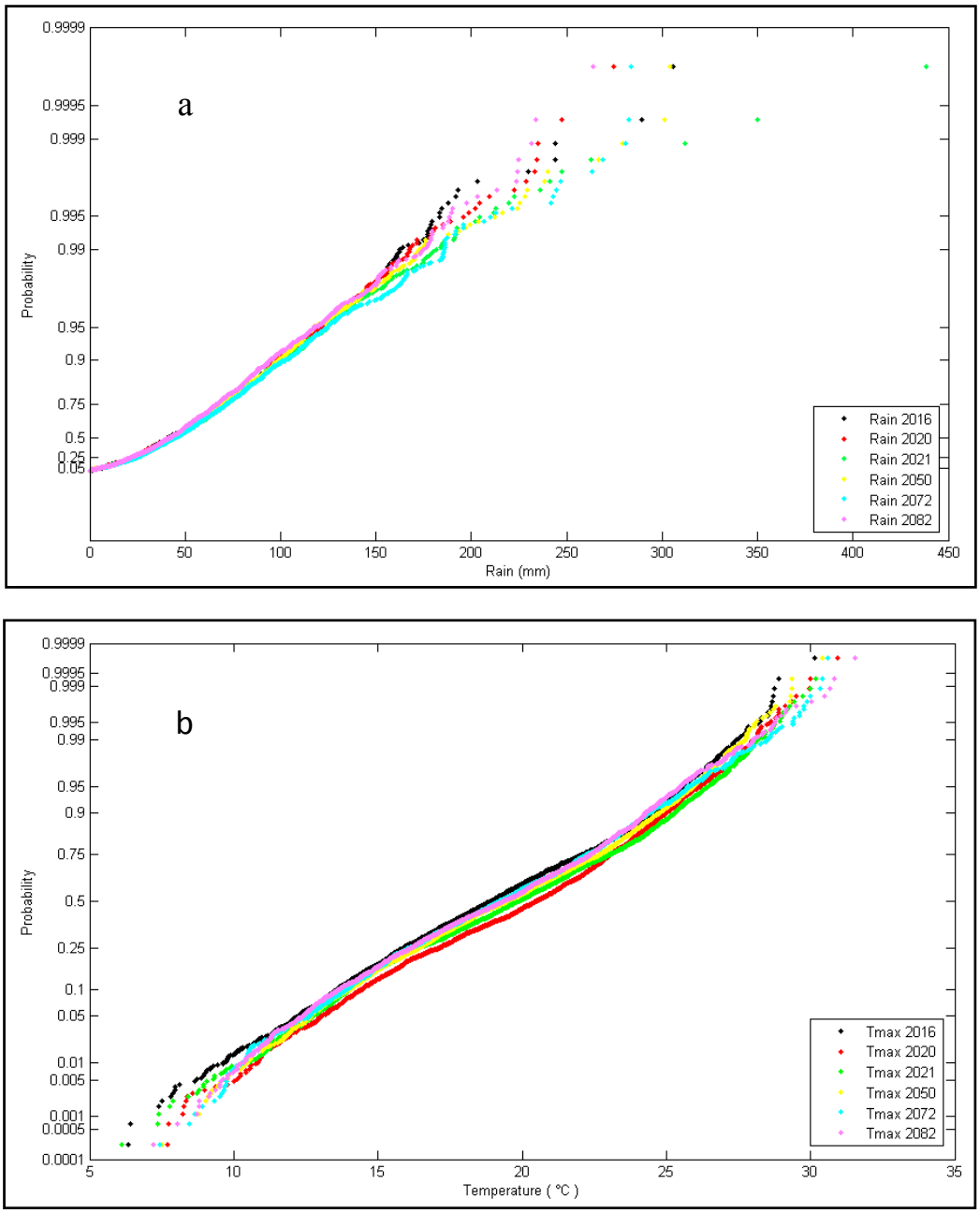

Figure 17: Probability plots for rain (a) and temperature (b) between 1- 31 May in the six selected years (average and five extreme years)

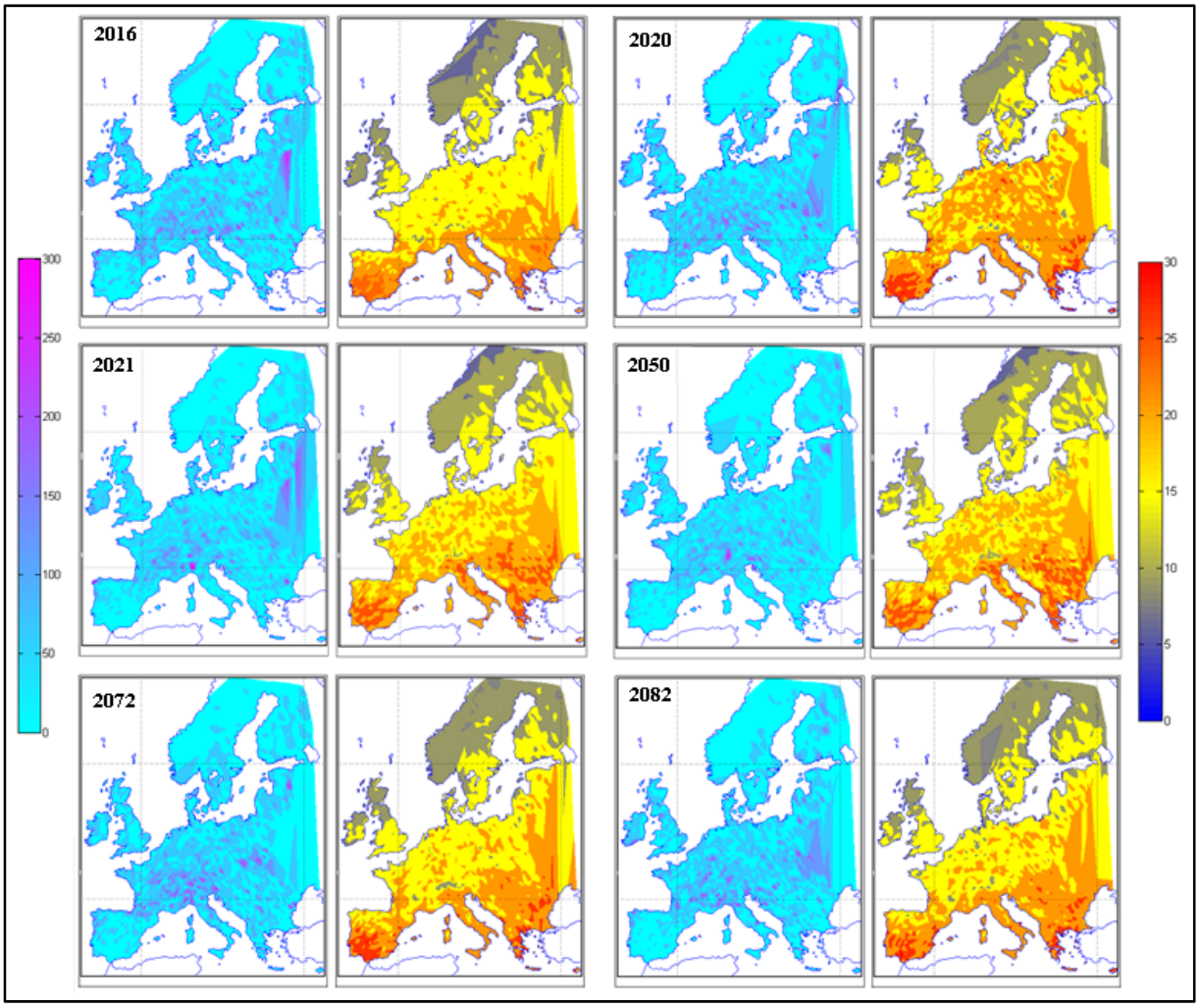

Figure 18: Maps drawn with mean data computed from 1-31 May of coldest (2016), warmest (2020), warm and dry (2021), average (2050), wettest (2072) and driest (2082) years; for each year, rain (mm) is reported on the left and Tmax (°C) on the right

Table 13: Mean amounts of rain predicted for the period 1-31 May in the selected years for each macroarea

Macroarea	Year	Mean rain	SE	Macroarea	Year	Mean rain	SE
IBERIA (Spain and Portugal)	All	42.2288	1.3187	**SCAND**	All	36.0594	0.6933
	2016	43.1167	2.2073		2016	37.278	1.8902
	2020	42.0351	2.3779		2020	35.238	1.5612
	2021	40.2682	2.3351		2021	36.7527	1.8386
	2050	38.6771	2.2183		2050	33.5932	1.7093
	2072	41.5784	2.2685		2072	36.9459	1.897
	2082	36.0216	1.8328		2082	35.2746	1.6172
ENG	All	54.0389	0.9519	**EEUROPA**	All	57.0924	0.7588
	2016	50.169	2.3945		2016	57.7083	2.5106
	2020	52.4813	2.1578		2020	58.7978	2.5707
	2021	56.7673	2.1916		2021	58.3656	2.2361
	2050	56.3368	2.2735		2050	60.2746	2.3402
	2072	50.4135	1.9708		2072	60.6112	2.4215
	2082	53.6491	2.3315		2082	60.2246	2.2736
ITALIA	All	46.9174	1.6514	**BALKGREE**	All	44.5652	0.8407
	2016	46.6612	2.6152		2016	44.4851	1.9919
	2020	43.0495	2.5005		2020	40.5098	1.8004
	2021	48.6009	3.4378		2021	45.2535	2.1804
	2050	45.8827	3.0014		2050	44.6684	2.0044
	2072	49.5523	2.9085		2072	45.2481	1.8545
	2082	49.2537	3.1048		2082	43.9411	1.9962
MIDDLEEU	All	66.365	1.1135	**BALTIC**	All	56.8196	1.7111
	2016	62.5224	2.7207		2016	57.9112	4.3775
	2020	65.3816	2.8087		2020	55.1387	3.838
	2021	66.2582	2.7318		2021	51.6062	4.8565
	2050	67.4741	2.9105		2050	59.7412	4.9688
	2072	71.391	3.0909		2072	59.0488	4.843
	2082	61.494	2.3194		2082	50.8012	4.0161

(SE): standard error

Table 14: Mean temperatures predicted for the period 1-31 May in the selected years

Macroarea	Year	Mean T	SE	Macroarea	Year	Mean T	SE
IBERIA	All	22.6936	0.1549	**SCAND**	All	14.2877	0.151
	2016	22.7466	0.1926		2016	13.8057	0.1866
	2020	23.2945	0.1938		2020	14.9276	0.2129
	2021	23.0157	0.1966		2021	14.5274	0.185
	2050	22.9118	0.2009		2050	14.5998	0.1705
	2072	22.5765	0.2127		2072	14.3224	0.1719
	2082	22.3933	0.1951		2082	14.2734	0.1676
ENG	All	14.8423	0.0856	**EEUROPA**	All	20.5792	0.0998
	2016	14.3091	0.1238		2016	19.9309	0.1212
	2020	15.3958	0.1446		2020	21.0556	0.1625
	2021	15.0137	0.1189		2021	21.6371	0.1646
	2050	15.1018	0.1416		2050	20.4529	0.153
	2072	14.8428	0.1302		2072	20.429	0.1543
	2082	15.0269	0.1285		2082	20.5392	0.1437
ITALIA	All	22.4689	0.1101	**BALKGREE**	All	23.1288	0.1031
	2016	22.2662	0.134		2016	22.7157	0.1411
	2020	22.5076	0.147		2020	23.1778	0.1322
	2021	22.9562	0.1599		2021	24.0876	0.1431
	2050	22.7338	0.1468		2050	23.207	0.1417
	2072	22.135	0.1411		2072	22.909	0.13
	2082	22.3328	0.1294		2082	23.1602	0.1256
MIDDLEEU	All	18.6843	0.0752	**BALTIC**	All	17.0756	0.1718
	2016	18.4435	0.1252		2016	16.482	0.2476
	2020	20.7642	0.1935		2020	18.8011	0.2724
	2021	19.1474	0.1597		2021	17.3302	0.3128
	2050	18.7552	0.1747		2050	17.796	0.3058
	2072	18.4191	0.1582		2072	17.7819	0.3371
	2082	18.2922	0.1613		2082	17.2618	0.309

(SE): standard error

3.2.2. Data selection for maize

Results from the statistical data analysis are shown in Figures 19, 20 and 21 and in Tables 15, 16 and 17.

The scatter plot of temperature and rain data from the period 15 June and 15 July (Figure 19), intended as the flowering period for maize, highlighted the years 2016, 2018, 2020, 2087 and 2088 as extreme years, with climatic conditions described in Table 15.

Basic statistics of temperature and rain data from the period between 15 June and 15 July (Table 15) showed that predicted rain ranged between 48.1 mm and 51.6 mm with a mean standard deviation of ±41.26 mm. Mean temperatures ranged between 25.3 mm and 26.5 mm, with a mean standard deviation of ±5.36. Simulations for the years 2016 and 2020 were expected to show extreme values, as seen for wheat, being cold and warm years with rain below and above mean, respectively.

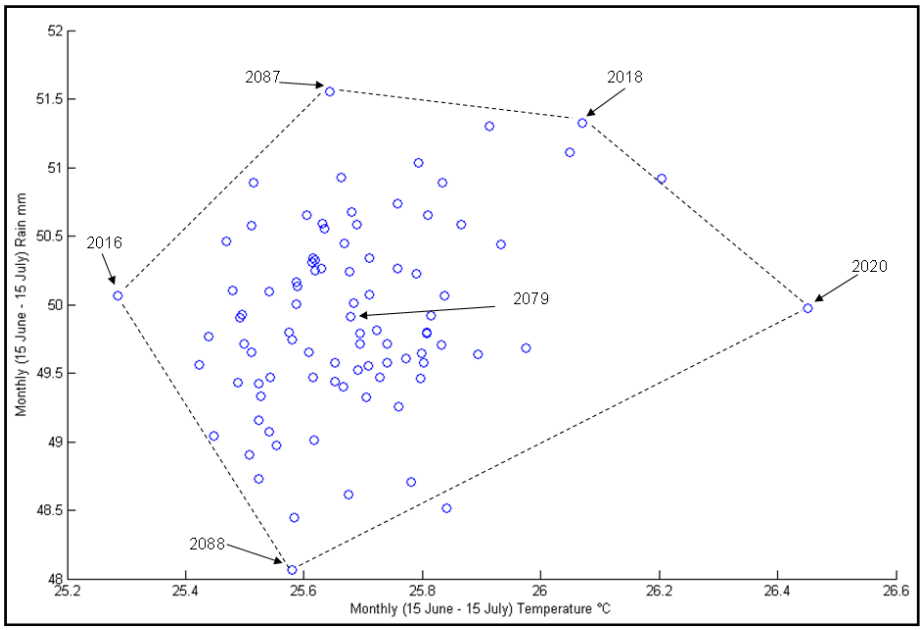

Figure 19: Scatter plot of meteorological data for the years 2011-2100: temperature and rain for the period 15 June – 15 July

When considering the macroareas, large differences were found in rainfall between 15 June and 15 July, with IBERIA and BALTIC as the driest and wettest macroareas, respectively (Table 16). The extreme years were not always the same in all the considered macroareas. A higher variability was also observed between macroareas than between years for T_{max}. The year 2016 was confirmed as the coldest, or very close to the coldest, and the year 2020 was almost always the warmest, or very close to the warmest (Table 17).

Therefore, the selected years seemed appropriate to show the variability expected within the next 100 years in rain and temperature. This variability was mapped and shown in Figure 21.

The probability plot (Figures 20a, b) was chosen to compare the probability of rain and temperature between 15 June and 15 July in the six different selected years. As for wheat data, the graph highlighted the limited range of data variation, according to the analysis performed at the European scale.

Table 15: Means of temperature and rain between 15 June – 15 July, for the whole set of years and for the selected years (average and five extreme years)

			Selected years					
		Set of 100 years	2016	2018	2020	2079	2087	2088
Rain (mm)	Mean	49.9	50.1	51.3	50.0	49.9	51.6	48.1
	Std	41.26	41.45	41.83	40.93	40.92	41.51	40.46
	SE	0.87	0.87	0.88	0.86	0.86	0.87	0.85
Temperature (°C)	Mean	25.6	25.3	26.1	26.4	25.7	25.6	25.6
	Std	5.36	5.35	5.06	5.08	5.33	5.53	5.48
	SE	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Year classification			**Coldest** Rainfall above average	Warm Wet	**Warmest**	**Average**	**Wettest**	**Driest** Temp. below average

(Std): standard deviations
(SE): standard error

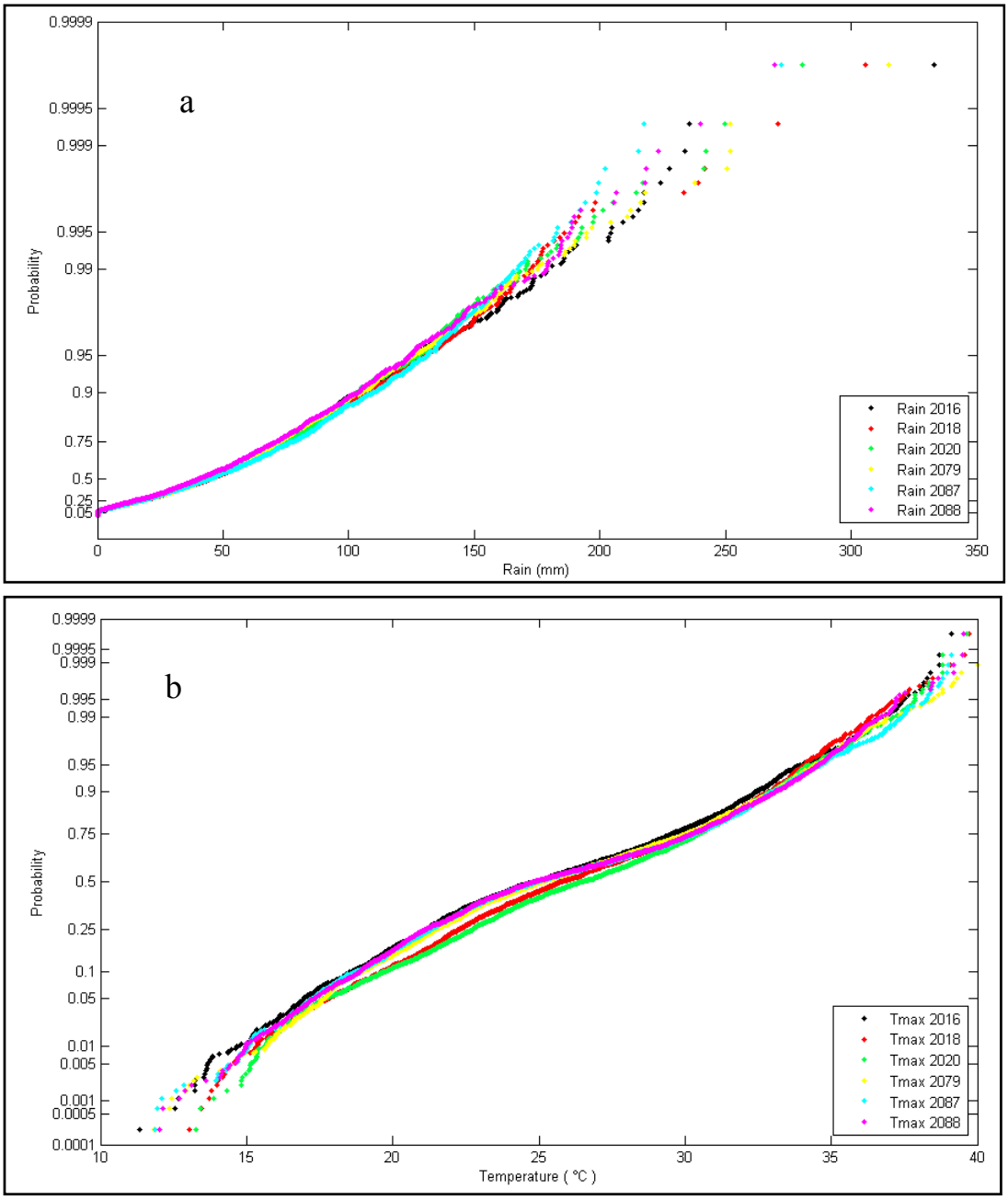

Figure 20: Probability plots for rain (a) and temperature (b) between 15 June - 15 July in the six selected years (average and five extreme years)

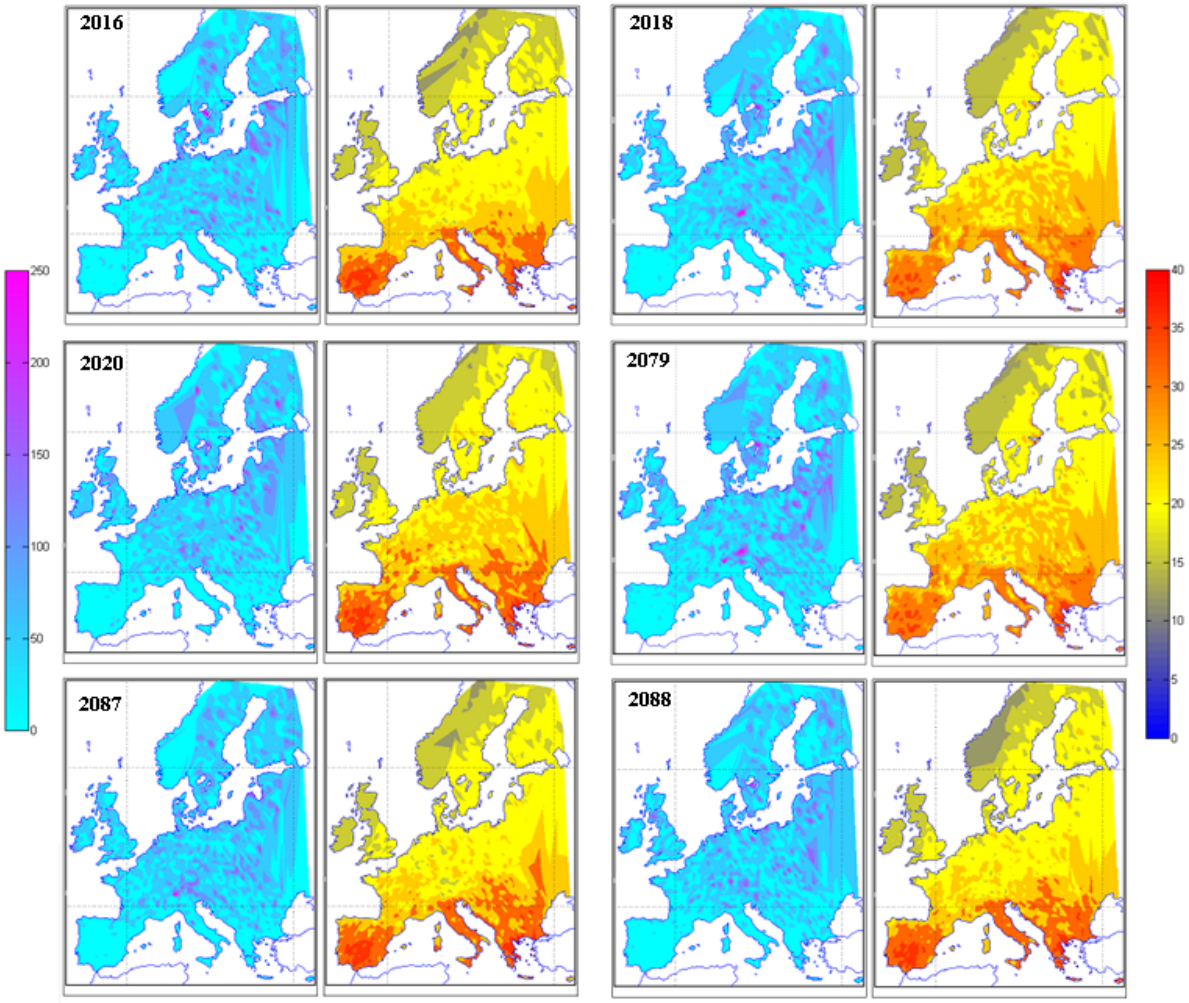

Figure 21: Maps drawn with mean data computed from 15 June to 15 July of coldest (2016), warm and wet (2018), hottest (2020), average (2079), wettest (2087) and driest (2088) years; for each year rain (mm) is reported on the left and T_{max} (°C) on the right

Table 16: Rain predicted for the period 15 June - 15 July in the selected years and for each macroarea

Macroarea	Year	Mean rain	SE	Macroarea	Year	Mean rain	SE
IBERIA	All	17.3393	0.6887	**SCAND**	All	63.9914	0.8149
	2016	18.9122	1.4167		2016	61.8137	3.0582
	2018	18.6261	1.5357		2018	67.6068	2.7164
	2020	17.2396	1.3366		2020	60.5429	2.8557
	2079	17.0412	1.2539		2079	63.321	2.5275
	2087	18.4673	1.5056		2087	63.8415	2.5583
	2088	14.1192	1.1376		2088	60.7912	2.636
ENG	All	59.8602	0.8505	**EEUROPA**	All	66.4636	0.8839
	2016	59.438	2.2615		2016	66.8065	2.4678
	2018	59.8058	2.383		2018	66.1873	2.33
	2020	67.6345	2.3558		2020	65.1359	2.4915
	2079	59.5626	2.3427		2079	70.1188	2.6093
	2087	61.4673	2.3507		2087	66.5399	2.4563
	2088	60.5105	2.5553		2088	63.6815	2.4695
ITALIA	All	26.9842	1.386	**BALKGREE**	All	32.624	1.0889
	2016	24.279	2.0012		2016	33.4991	2.0291
	2018	26.6584	2.2631		2018	33.9266	2.0267
	2020	24.5981	2.1483		2020	29.8209	1.7712
	2079	27.3238	2.3088		2079	39.3361	2.3538
	2087	27.3248	2.2315		2087	33.7028	2.0618
	2088	24.4907	2.104		2088	31.732	1.8371
MIDDLEEU	All	70.1943	0.8899	**BALTIC**	All	82.7081	1.595
	2016	69.0945	2.5561		2016	87.6863	5.2864
	2018	71.8239	3.0032		2018	90.1325	5.8188
	2020	68.3572	2.5372		2020	89.8788	4.3737
	2079	70.2975	2.6067		2079	78.3887	4.5871
	2087	73.8075	2.7983		2087	85.5425	4.9789
	2088	70.1721	2.9358		2088	81.685	4.4341

(SE): standard error

Table 17: Temperatures predicted for the period 15 June - 15 July in the selected years and for each macroarea

Macroarea	Year	Mean T	SE	Macroarea	Year	Mean T	SE
IBERIA	All	31.4547	0.1908	**SCAND**	All	20.1504	0.1401
	2016	31.4072	0.2252		2016	19.9506	0.1814
	2018	31.0347	0.2175		2018	21.0937	0.1978
	2020	31.4246	0.2219		2020	21.5361	0.2091
	2079	31.6807	0.2368		2079	20.2956	0.1776
	2087	31.5838	0.2394		2087	20.0582	0.1861
	2088	31.6989	0.2109		2088	19.882	0.1808
ENG	All	18.4591	0.129	**EEUROPA**	All	26.295	0.1569
	2016	18.1682	0.1601		2016	25.8498	0.1635
	2018	18.6982	0.1636		2018	26.5588	0.1628
	2020	19.0111	0.1875		2020	27.2613	0.175
	2079	18.5182	0.1586		2079	26.2443	0.1856
	2087	18.3412	0.1532		2087	26.4496	0.2133
	2088	18.3428	0.1607		2088	26.1992	0.1992
ITALIA	All	30.458	0.1431	**BALKGREE**	All	31.2915	0.1205
	2016	30.2852	0.1637		2016	30.7576	0.1512
	2018	30.4164	0.1883		2018	31.1548	0.1673
	2020	30.4632	0.1751		2020	31.3555	0.1523
	2079	30.3525	0.1849		2079	31.0114	0.1775
	2087	30.6734	0.1844		2087	31.5802	0.1647
	2088	30.6656	0.1759		2088	31.4824	0.1537
MIDDLEEU	All	23.632	0.0771	**BALTIC**	All	22.3029	0.0935
	2016	23.4514	0.1484		2016	21.4263	0.1972
	2018	24.6668	0.1393		2018	22.629	0.1813
	2020	25.6452	0.1886		2020	23.2217	0.214
	2079	23.5909	0.1659		2079	22.3131	0.2141
	2087	23.4573	0.1673		2087	22.0522	0.2246
	2088	23.3541	0.1753		2088	21.6049	0.2447

(SE): standard error

3.2.3. Data selection for rice

Results from the statistical data analysis are shown in the Figures 22, 23 and 24 and in Tables 18, 19 and 20.

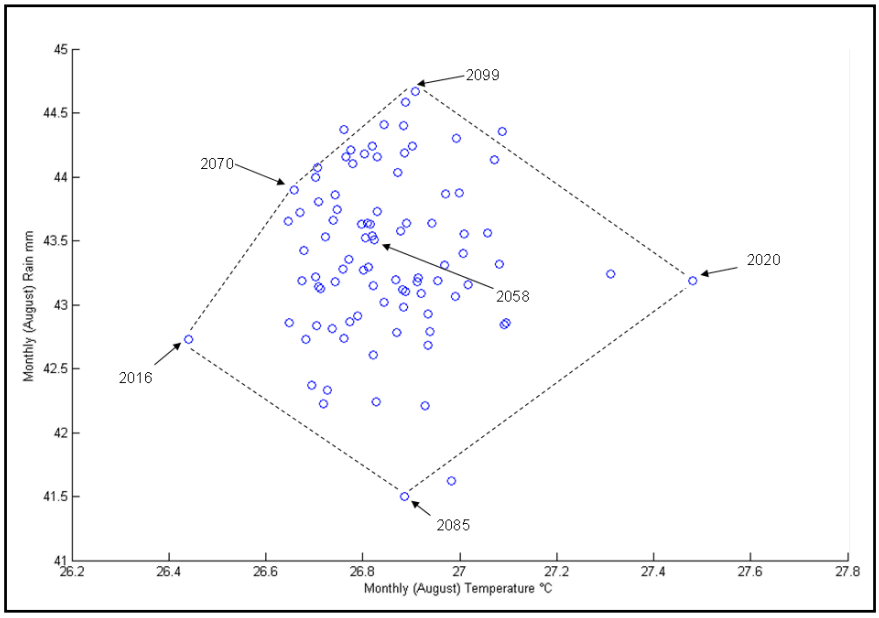

Figure 22: Scatter plot of meteorological data for the years 2011-2100: temperature and rain for the periods 15 June – 15 July and 1 August – 31 August

The scatter plot of temperature and rain data of the month of August (Figure 22), intended as the flowering period for rice, highlighted the years 2016, 2020, 2070, 2085 and 2099 as extreme years and 2058 as the average year, with climatic conditions described in Table 18.

Basic statistic on temperature and rain data from the month of August (Table 18) showed that predicted rain ranged between 41.5 mm and 44.7 mm with a mean standard deviation of ±37.59 mm. Mean temperatures ranged between 26.4 °C and 27.5 °C, with a mean standard deviation of ±5.64 °C. Simulations for the years 2016 and 2020 were expected to show extreme values, as seen for wheat, being a cold and a warm year with rain above and below mean, respectively.

As highlighted in wheat and maize, the probability plot for rice (Figures 23a, b) showed a limited range of variation, according to the analysis performed at the European scale.

When considering macroareas, large rainfall differences were found in August, with IBERIA and BALTIC, the dryer and wettest macroareas, respectively (Table 19), as reported for the maize flowering period. Extreme years were not always the same in all the considered macroareas. A higher variability was observed between macroareas than between years for T_{max}. The year 2016 was confirmed as the coldest, or very close to the coldest, and the year 2020 was almost always the warmest, or very close to the warmest (Table 20).

Therefore, the selected years seemed appropriate to show the variability expected within the next 100 years in rain and temperature. This variability was mapped and shown in Figure 22.

Six years, that summarise the upper and lower case, the average and three years characterized by rain or temperature above/below average, have been selected.

The results for the four different short-term periods, as expected, highlighted a very limited range of variability in the 100 simulated years set, due to different climatic conditions that characterize the different European macroareas and due to a strong difference in projected precipitation change

between Northern (getting wetter) and Southern (becoming drier) European countries, especially during summer.

At the EU scale, slight variations were found between dry and wet conditions (i.e. a few millimetres of rain difference) and between warm and cold short-term periods (i.e. a just over 1°C difference).

Table 18: Means of temperatures and rain between 1–30 August, for the whole set of years and for the selected years (average and five extreme years)

			Selected years					
		Set of 100 years	2016	2020	2058	2070	2085	2099
Rain (mm)	Mean	43.4	42.7	43.2	43.5	43.9	41.5	44.7
	Std	37.59	38.01	36.96	37.76	38.49	36.58	40.18
	SE	0.79	0.81	0.78	0.79	0.81	0.77	0.85
Temperature (°C)	Mean	26.8	26.4	27.5	26.8	26.7	26.9	26.9
	Std	5.64	5.63	5.49	5.49	5.70	5.66	5.65
	SE	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Year classification			Coldest Rain below average	Warmest Rain below average	Average	Temp below average, Rain above average	Driest	Wettest Temp above average

(Std): standard deviations
(SE): standard error

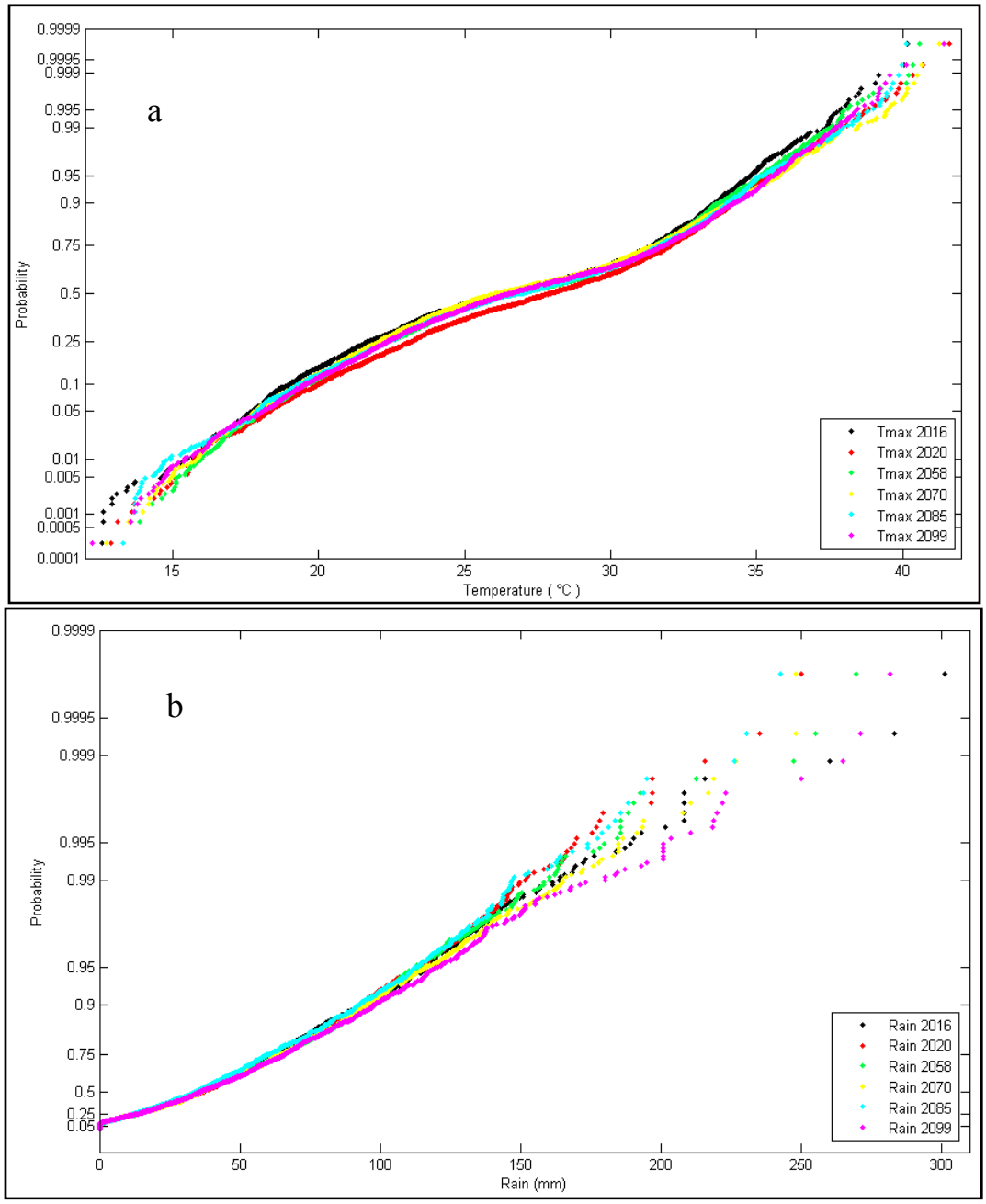

Figure 23: Probability plot of rain (a) and temperature (b) between 1-31 August in the selected years (average and five extreme years)

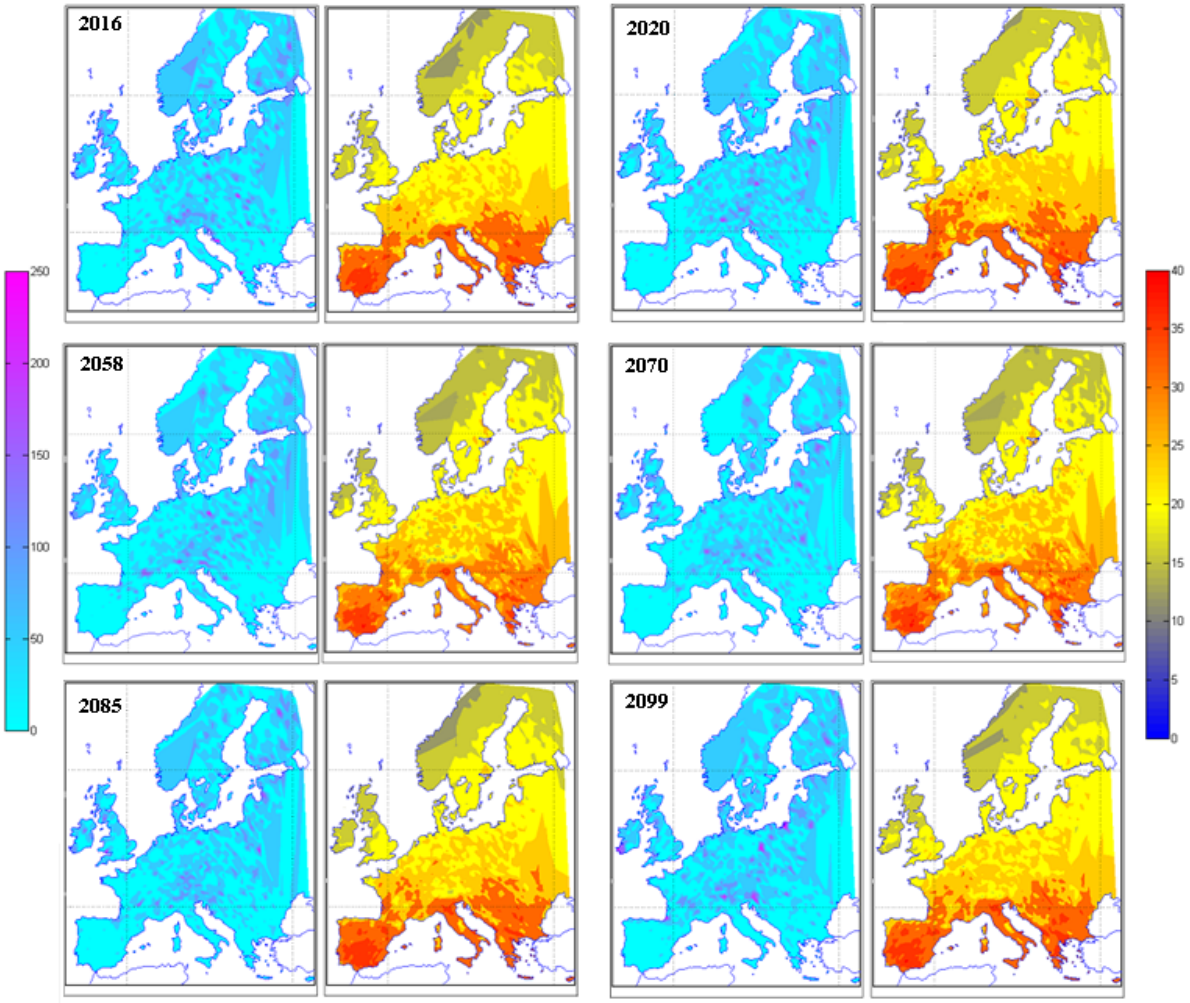

Figure 24: Maps drawn with mean data computed from 1 August to 31 August of coldest (2016), hottest (2020), average (2058), year with temperature below average and rain above average (2070), wettest (2099) and driest (2085) years; for each year, rain (mm) is reported on the left and Tmax (°C) on the right

Table 19: Rain predicted for the period 1 - 31 August in the selected years for each macroarea

Macroarea	Year	Mean rain	SE	Macroarea	Year	Mean rain	SE
IBERIA	All	15.5044	0.8344	**SCAND**	All	51.0715	0.6134
	2016	15.3755	1.4849		2016	50.7068	1.9906
	2020	12.9273	1.3599		2020	54.3546	2.12
	2058	16.3759	1.6348		2058	48.3732	1.8911
	2070	14.3649	1.438		2070	52.7771	2.248
	2085	16.482	1.4865		2085	50.9683	2.1811
	2099	15.7114	1.5782		2099	53.82	2.1787
ENG	All	59.1624	1.1317	**EEUROPA**	All	51.8606	0.6966
	2016	60.1129	2.7531		2016	48.6663	2.3359
	2020	56.1912	2.4272		2020	52.4464	2.1871
	2058	61.3649	2.4587		2058	51.8971	2.2724
	2070	66.0515	3.0406		2070	54.2243	2.2622
	2085	57.4433	3.1888		2085	49.4942	2.0759
	2099	65.1368	3.2729		2099	51.5562	2.3111
ITALIA	All	30.7521	1.1137	**BALKGREE**	All	28.3201	0.8182
	2016	30.221	2.5463		2016	30.3364	2.2239
	2020	30.0701	2.2363		2020	28.5915	1.7223
	2058	32.3495	2.5393		2058	29.163	1.8775
	2070	30.55	2.5578		2070	27.95	1.7785
	2085	28.0678	2.2422		2085	28.0487	1.6739
	2099	26.4364	2.1446		2099	26.8278	1.7126
MIDDLEEU	All	60.2404	1.0087	**BALTIC**	All	69.3632	1.223
	2016	58.304	2.7192		2016	64.3	3.6712
	2020	62.3881	2.6916		2020	70.4625	4.2601
	2058	59.91	2.6772		2058	70.8875	4.2982
	2070	61.4159	2.4067		2070	69.4737	4.5649
	2085	56.9194	2.4904		2085	62.6163	4.4718
	2099	60.3159	2.7787		2099	69.685	3.8173

(SE): standard error

Table 20: Temperature predicted for the period 1 - 31 August in the selected years for each macroarea

Macroarea	Year	Mean T	SE	Macroarea	Year	Mean T	SE
IBERIA	All	33.2562	0.17	**SCAND**	All	20.3284	0.1759
	2016	32.8579	0.1872		2016	19.75	0.2113
	2020	33.3147	0.1937		2020	21.3043	0.2235
	2058	33.0669	0.1884		2058	20.6372	0.1966
	2070	33.1831	0.2209		2070	20.0618	0.201
	2085	33.298	0.2023		2085	20.5002	0.1993
	2099	33.4362	0.1926		2099	20.4259	0.1892
ENG	All	19.7506	0.144	**EEUROPA**	All	27.4688	0.1731
	2016	19.4858	0.1396		2016	27.2949	0.1857
	2020	20.2699	0.2041		2020	28.2785	0.1746
	2058	19.7699	0.1581		2058	27.649	0.1861
	2070	19.6116	0.1464		2070	26.9908	0.1967
	2085	19.4341	0.1758		2085	27.7421	0.1997
	2099	19.7688	0.1786		2099	27.2346	0.1972
ITALIA	All	32.0764	0.1255	**BALKGREE**	All	32.511	0.0993
	2016	31.6454	0.141		2016	32.1544	0.1143
	2020	31.8639	0.1494		2020	32.2863	0.1368
	2058	31.9871	0.1392		2058	32.2489	0.1263
	2070	32.0036	0.1538		2070	32.4713	0.1394
	2085	31.9661	0.1637		2085	32.3945	0.1299
	2099	32.1517	0.1666		2099	32.5328	0.1363
MIDDLEEU	All	24.9767	0.0804	**BALTIC**	All	22.9096	0.0907
	2016	24.3751	0.1498		2016	22.0866	0.1505
	2020	26.2992	0.1824		2020	23.3268	0.1708
	2058	24.9058	0.136		2058	22.8963	0.1581
	2070	24.4125	0.1377		2070	22.8302	0.1858
	2085	25.2562	0.1575		2085	22.9243	0.2024
	2099	25.2025	0.1467		2099	23.0656	0.1974

(SE): standard error

3.2.4. Data selection

The selected years covered various weather conditions and not just extremes. Therefore, the analysis was performed at a reduced scale, selecting eight macroareas with similar climate conditions and latitudes. As expected, the years were classified according to their climatic conditions and their deviation from the mean by an analysis at the European scale, but showed different situations at the macroarea scale. This was not interpreted as a limiting factor, but as an opportunity to initialize the models taking into account the real effects of climate change and the different weather conditions that characterize Europe.

The results of the data analysis showed that by running the models for the selected years, the crops responded to a wide range of weather conditions which was analysed.

3.3. Crop phenology in different climate change scenarios

This section describes the results of the statistical analyses of the shifts in crop phenology due to climate change for wheat and maize, as based on the JRC/MARS crop models.

3.3.1. Wheat phenology

For the reference situation (i.e. for the current climate), the majority of the median flowering dates of wheat grown within Europe was estimated to be between days 150-180 (Figure 25). In the +2 °C scenario, the median flowering date was generally early (i.e. most of the flowering dates were between days 142-174). In the +5 °C scenario, wheat flowering was estimated to be much more advanced (i.e. the majority of the medians was between days 125-150; Figure 25). Differences in median flowering dates, per grid (based on 100 runs per grid), between two climatic scenarios were shown in Figure 25. The shift in median flowering date - from the reference to the +2 °C scenario - was estimated to be 10 days in most grids, ranging from zero to 17 days between grids. In the +5 °C scenario (relative to the reference situation), the shift was much higher, ranging from 10 to more than 40 days, but most of the median flowering dates were estimated to be advanced by 22-28 days. Figure 26 presents median harvest dates of wheat grown in Europe in the three different climatic scenarios. Just like in Figure 25, medians are based on 100 runs per grid; the histogram presents median values for the different grids in Europe. The median harvest dates for wheat were not found to change much in the +2 °C scenario (relative to the reference situation). The difference between medians was mostly around 10 days, varying from zero to 25 days earlier harvest. However, in the +5 °C scenario, the median harvest date was estimated to be much more advanced; in most of the study area, harvest was estimated to be before day 200 as compared to day 230 in the reference situation (Figure 26). In general, time elapsed between median flowering and median harvesting dates (in number of days) decreased, generally down to 5 days. However, for some grids, it could even increase up to one week (results not shown). Maps with estimated flowering and harvest dates of wheat throughout Europe in the two different scenarios, (i.e. the current and +5 °C scenarios), in the year 2050, were presented in Figures 27 and 28 for flowering and harvest dates, respectively.

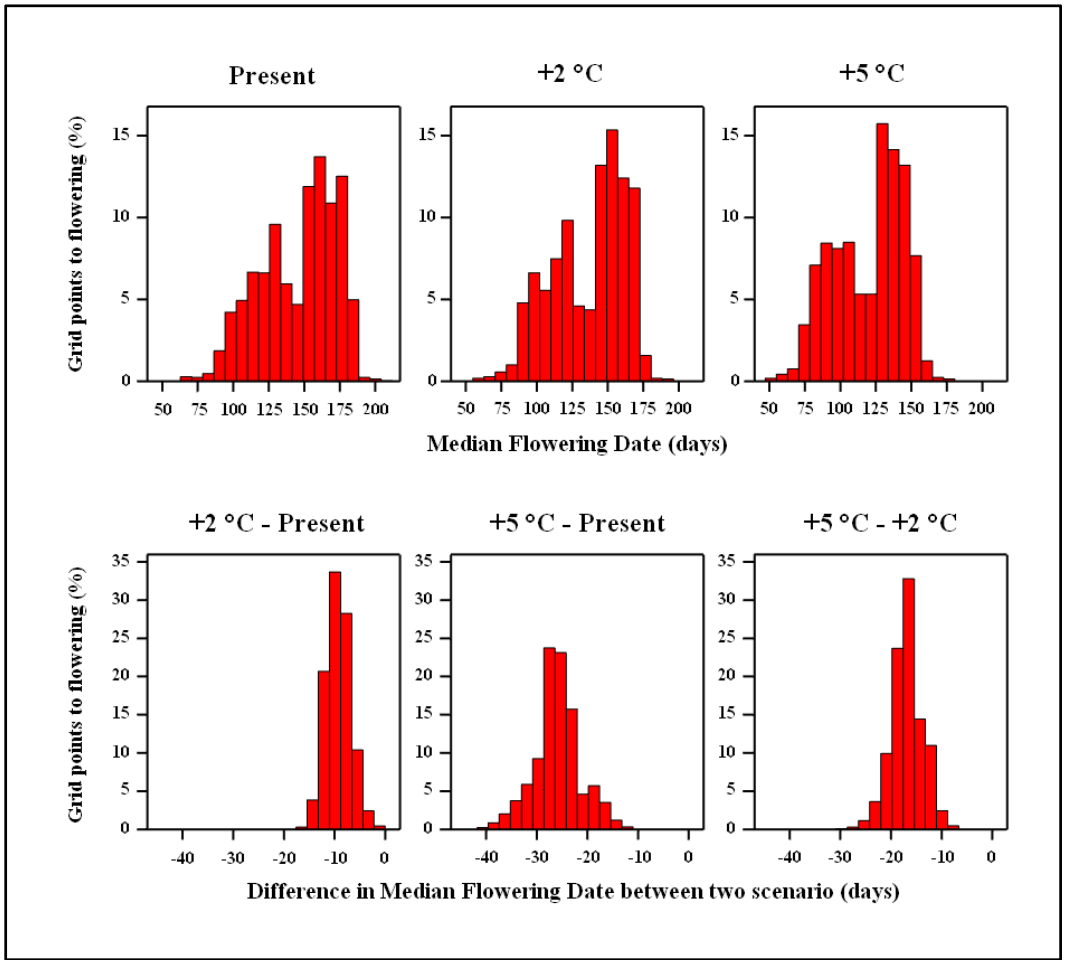

Figure 25: Distribution of median flowering dates (based on 100 runs) of wheat over Europe in the reference situation, the + 2 °C and + 5 °C scenarios, as well as differences between the two scenarios

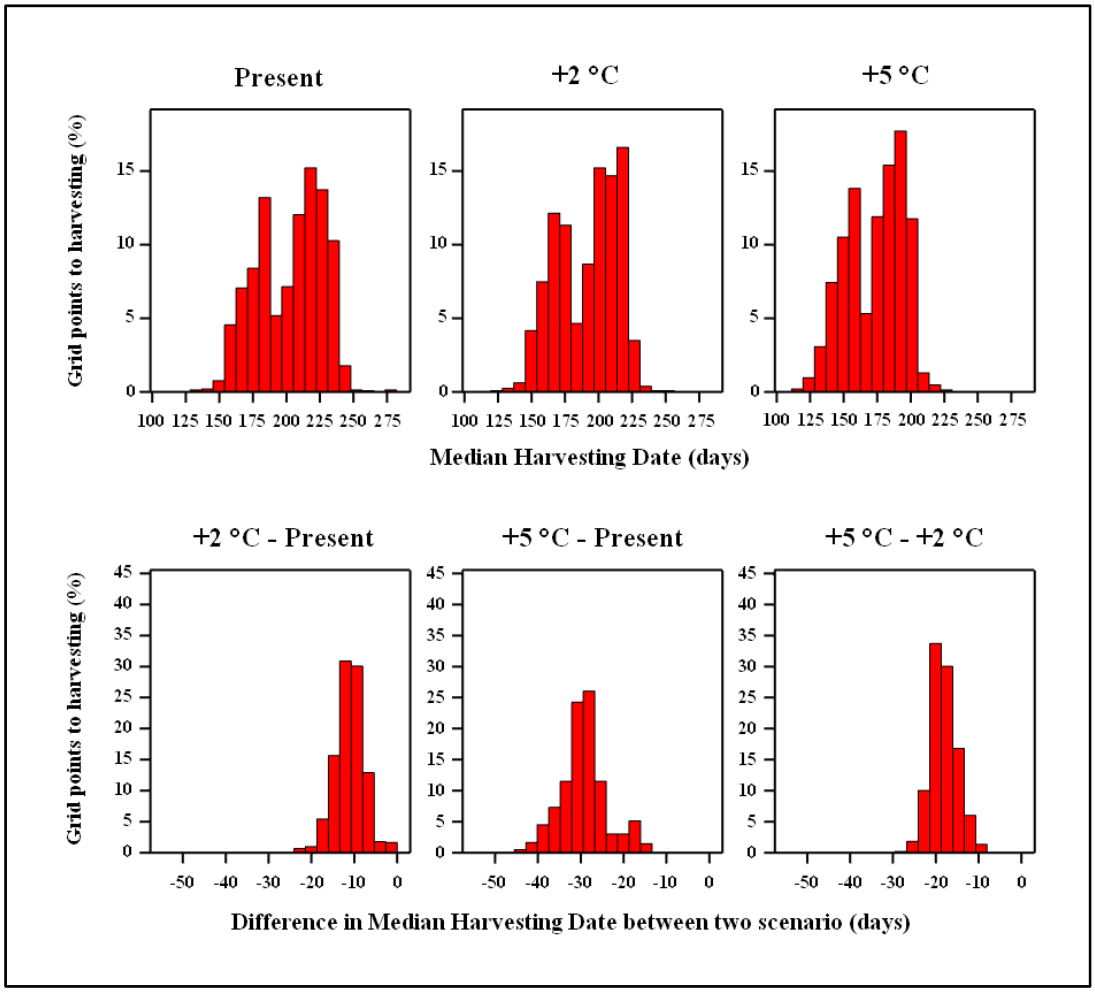

Figure 26: Distribution of median harvest dates (based on 100 runs) of wheat over Europe in the reference situation, the + 2 °C scenario and + 5 °C scenarios, as well as differences between the two scenarios

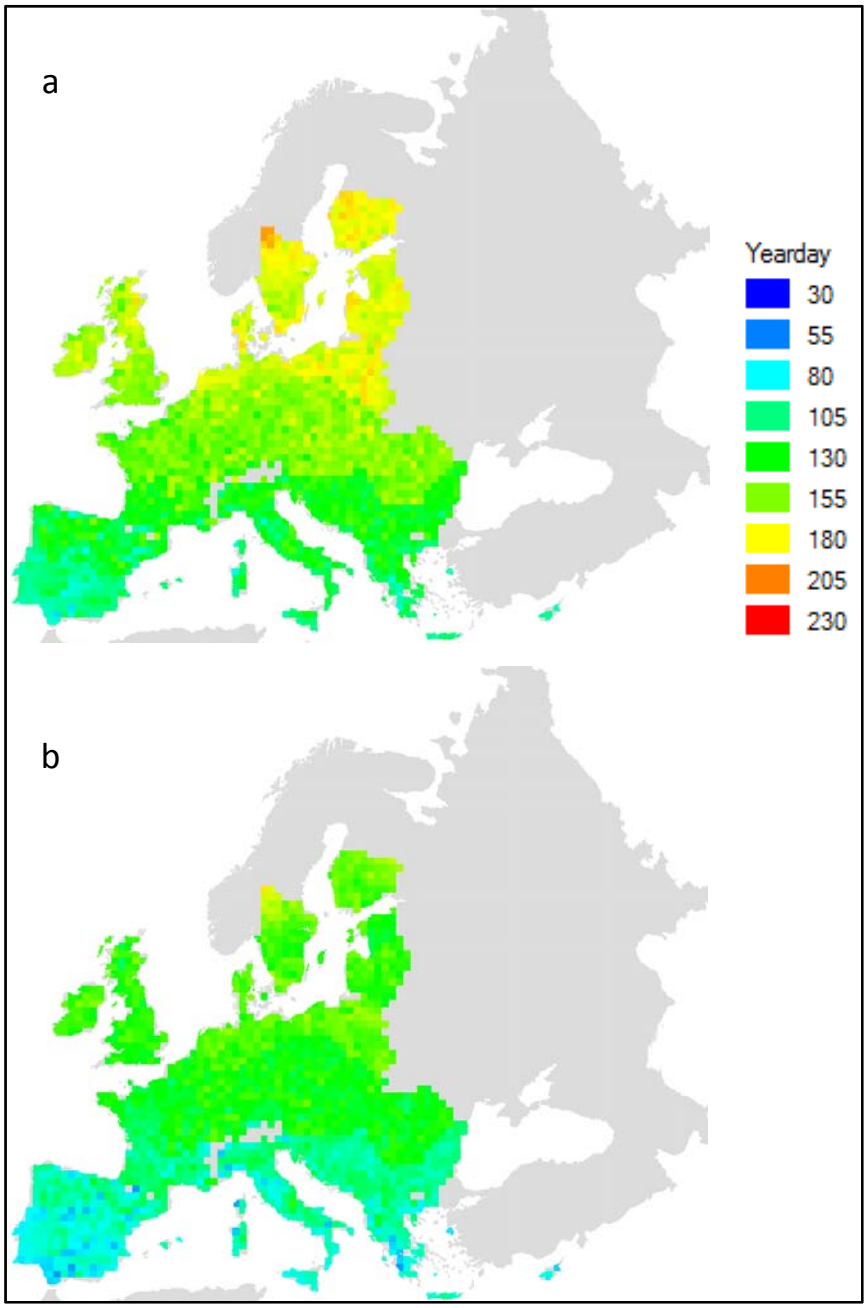

Figure 27: Wheat flowering date (ordinal date) in 2050 in the reference (a) and +5 °C (b) scenarios

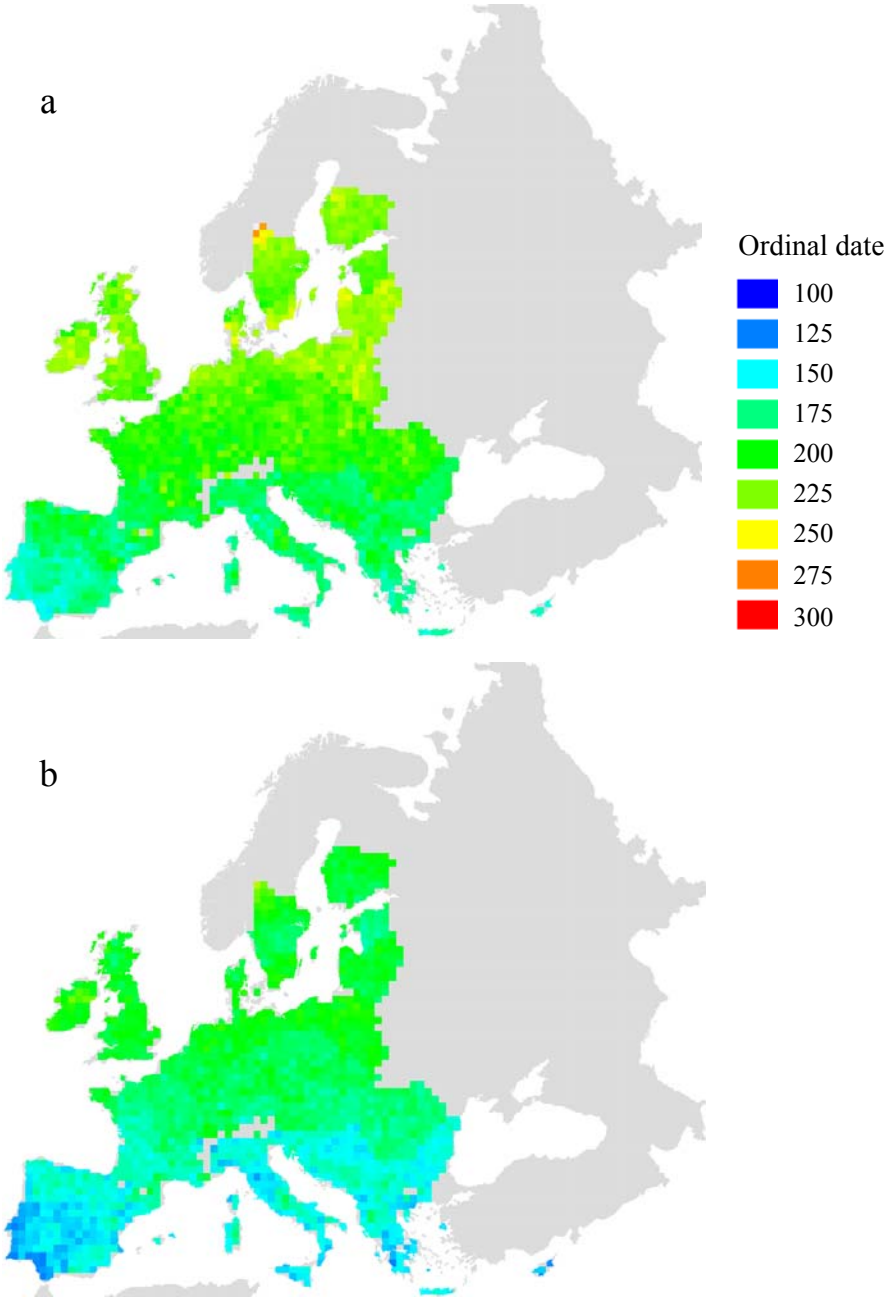

Figure 28: Wheat harvest date (ordinal date) in 2050 in the reference (a) and + 5 °C (b) scenarios

3.3.2. Maize phenology

The distribution of median flowering dates of maize cultivated in the different grids in Europe is presented in Figure 29. The median flowering date in the reference situation was mostly at day 185, but there was a lot of variation. In the + 2 °C and + 5 °C scenarios, median flowering dates were at 180 and 175 days, respectively, in most grids. However, just like in the reference situation, the ranges of the median flowering dates were wide. Looking at the shifts in median flowering dates in the + 2 °C scenario (relative to the reference situation), it was estimated to be 5 days earlier in most grids, having a quite small interval from zero up to 9 days earlier (Figure 29). In the + 5 °C situation, median

flowering dates were estimated to be 5 up to 20 days earlier in the season as compared to the reference situation (Figure 29).

The distribution of the median harvest dates of maize cultivated throughout Europe is presented in Figure 30. The median harvest date in the reference situation was at about day 220. In the +5 °C scenario, the median harvest date was advanced by 20 days in most of the grids. However, shifts in harvest dates ranged from 5 to 32 days earlier. In the +2 °C scenario (relative to the reference situation), shifts ranged from 5 to 18 days earlier (Figure 30).

Maps with the estimated flowering and harvest dates throughout Europe in the two different scenarios, (i.e. the current and +5 °C scenarios), in the year 2050, were presented in Figures 31 and 32 for flowering and harvest dates, respectively.

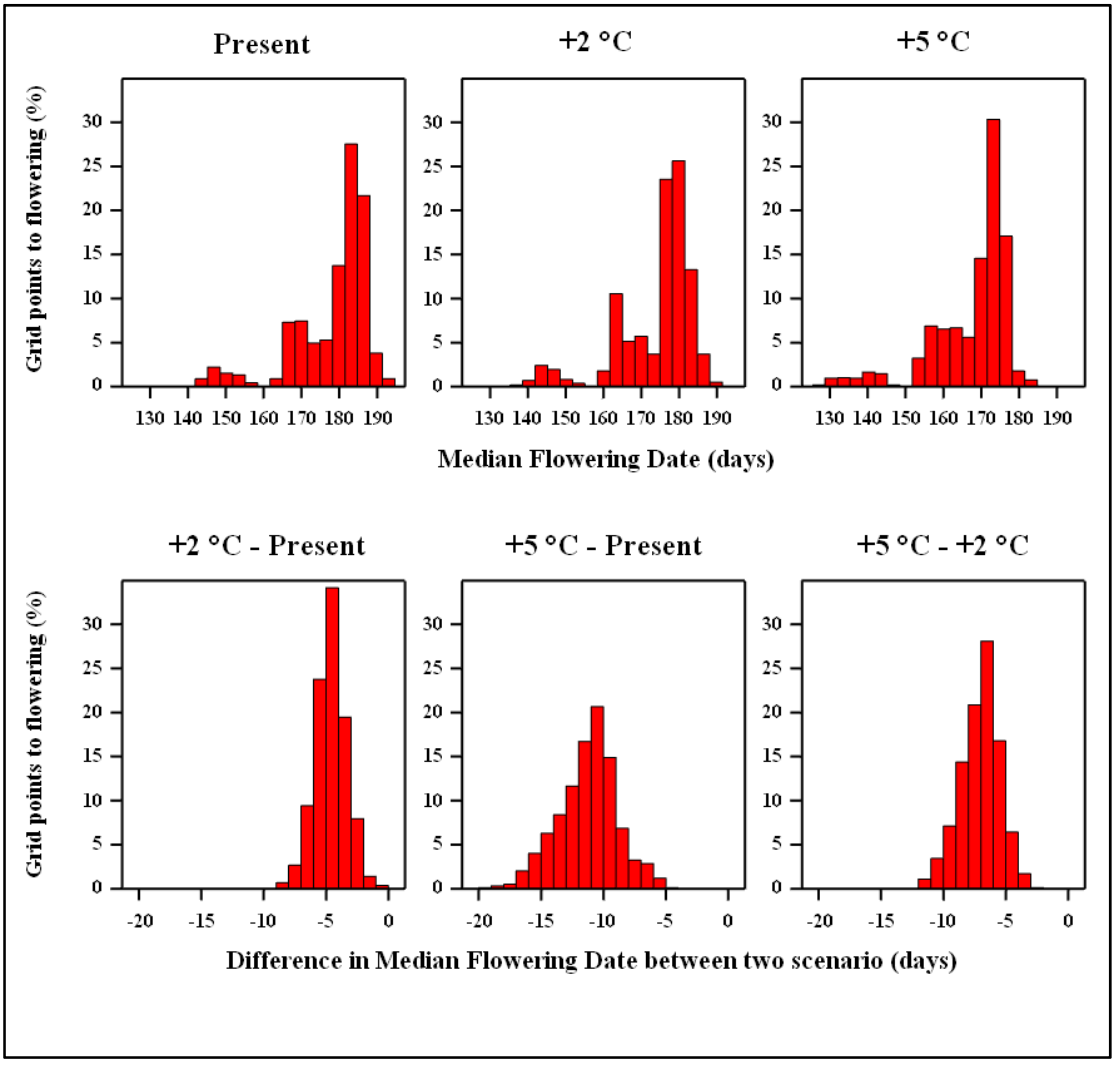

Figure 29: Distribution of median flowering dates (based on 100 runs) of maize over Europe in the reference situation, the +2 °C scenario and +5 °C scenarios, as well as differences between the two scenarios

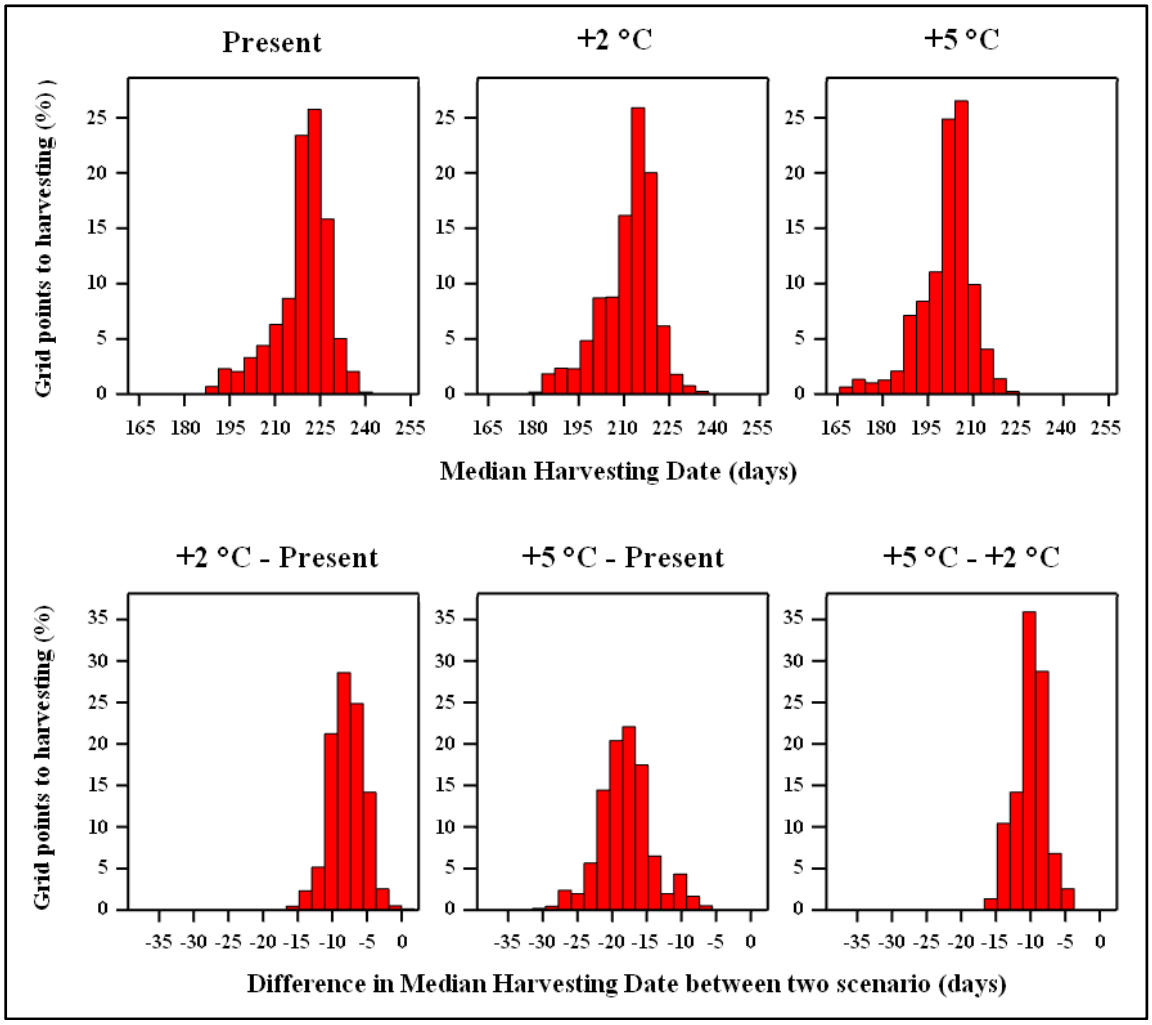

Figure 30: Distribution of median harvest dates (based on 100 runs) of maize over Europe in the reference situation, the + 2 °C scenario and + 5 °C scenarios, as well as differences between the two scenarios

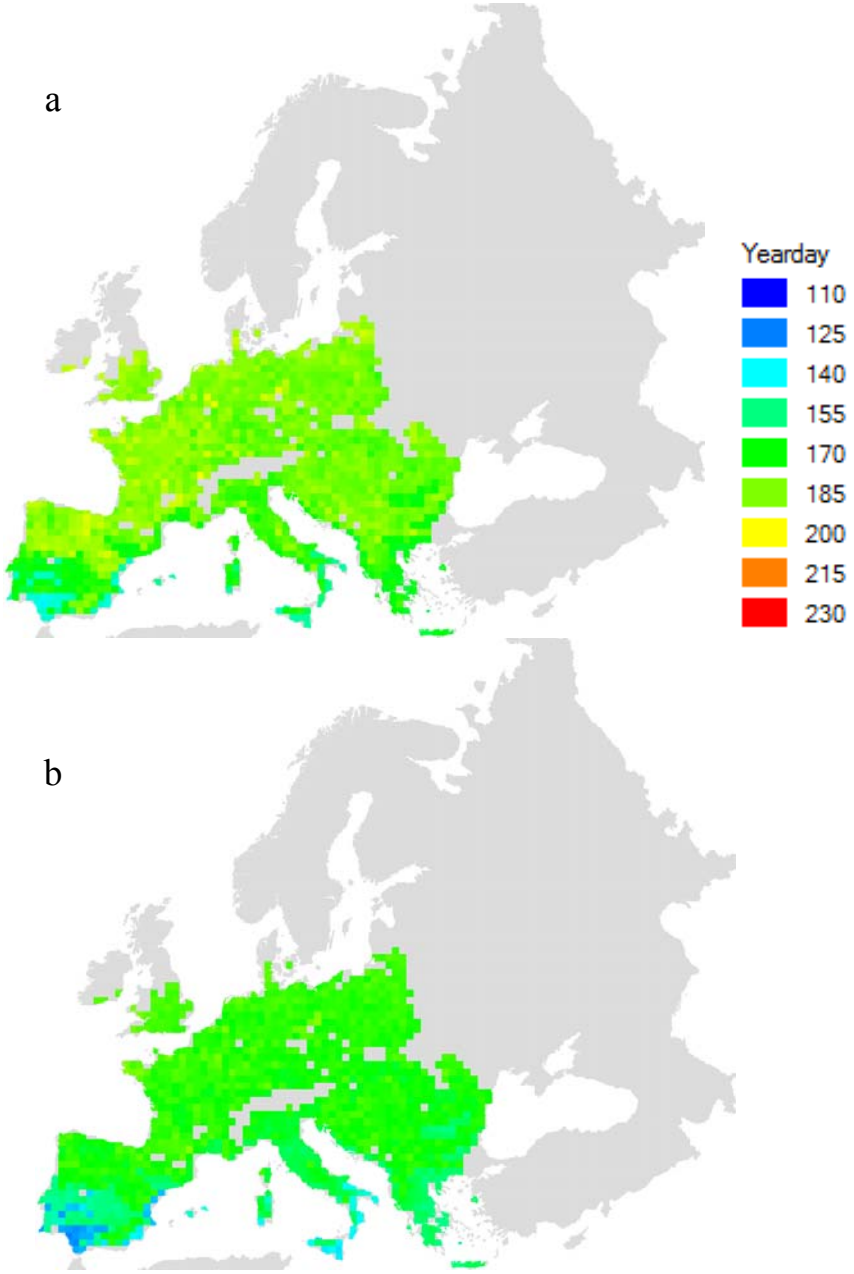

Figure 31: Maize flowering date (ordinal date) in 2016 in the reference (a) and +5 °C (b) scenarios

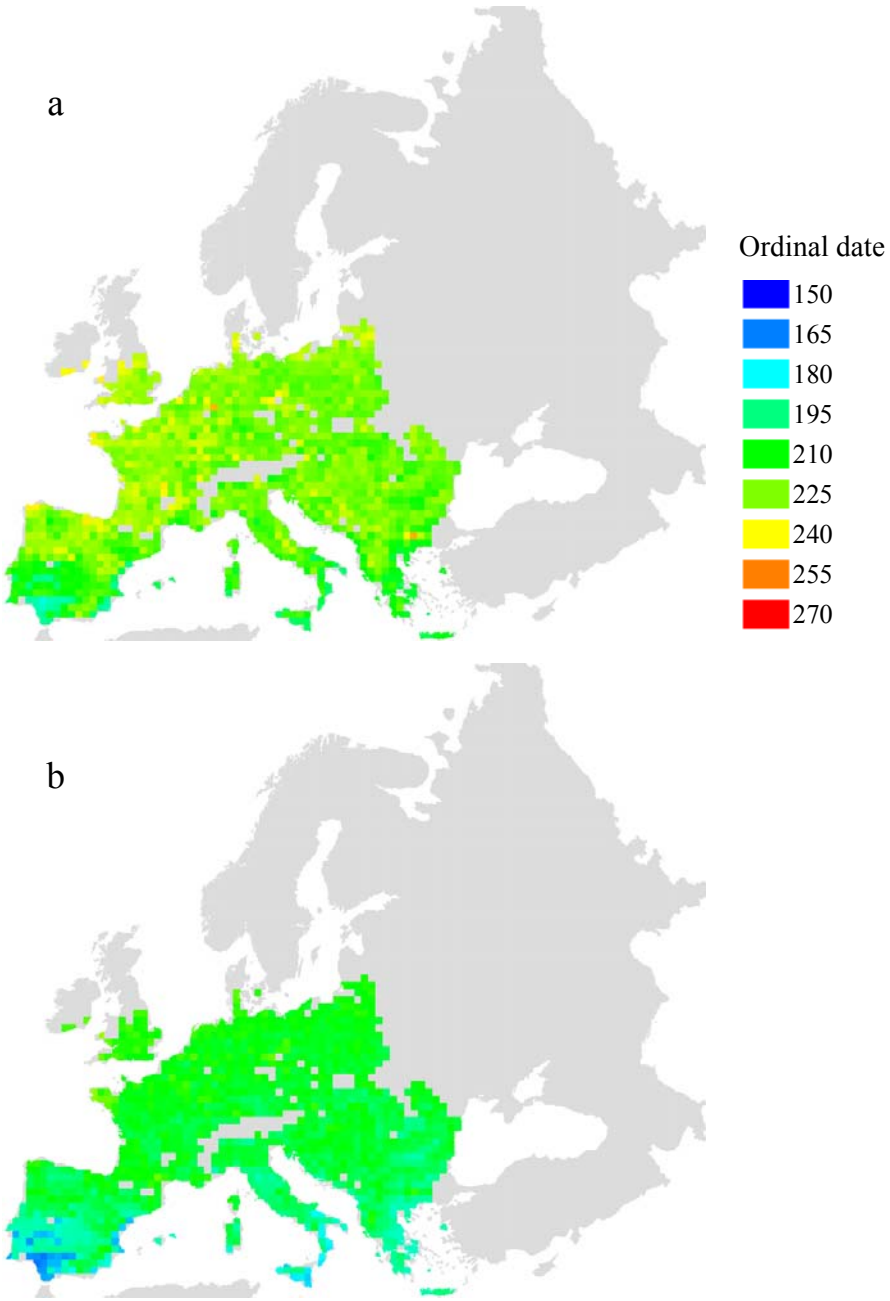

Figure 32: Maize harvest date (ordinal date) in 2016 in the reference (a) and + 5 °C (b) scenarios

3.3.3. Rice phenology

The distribution of the median flowering dates of rice cultivated in the different grids in Europe is presented in Figure 33. The median flowering date in the reference situation (present scenario and the six selected years) was mostly at day 223, but there was a lot of variation. In the + 2 °C and + 5 °C scenarios, median flowering dates were at days 217 and 211, respectively, in most grids. However, just like in the reference situation, the ranges of the median flowering dates were wide and increased in the + 2 °C and + 5 °C scenarios.

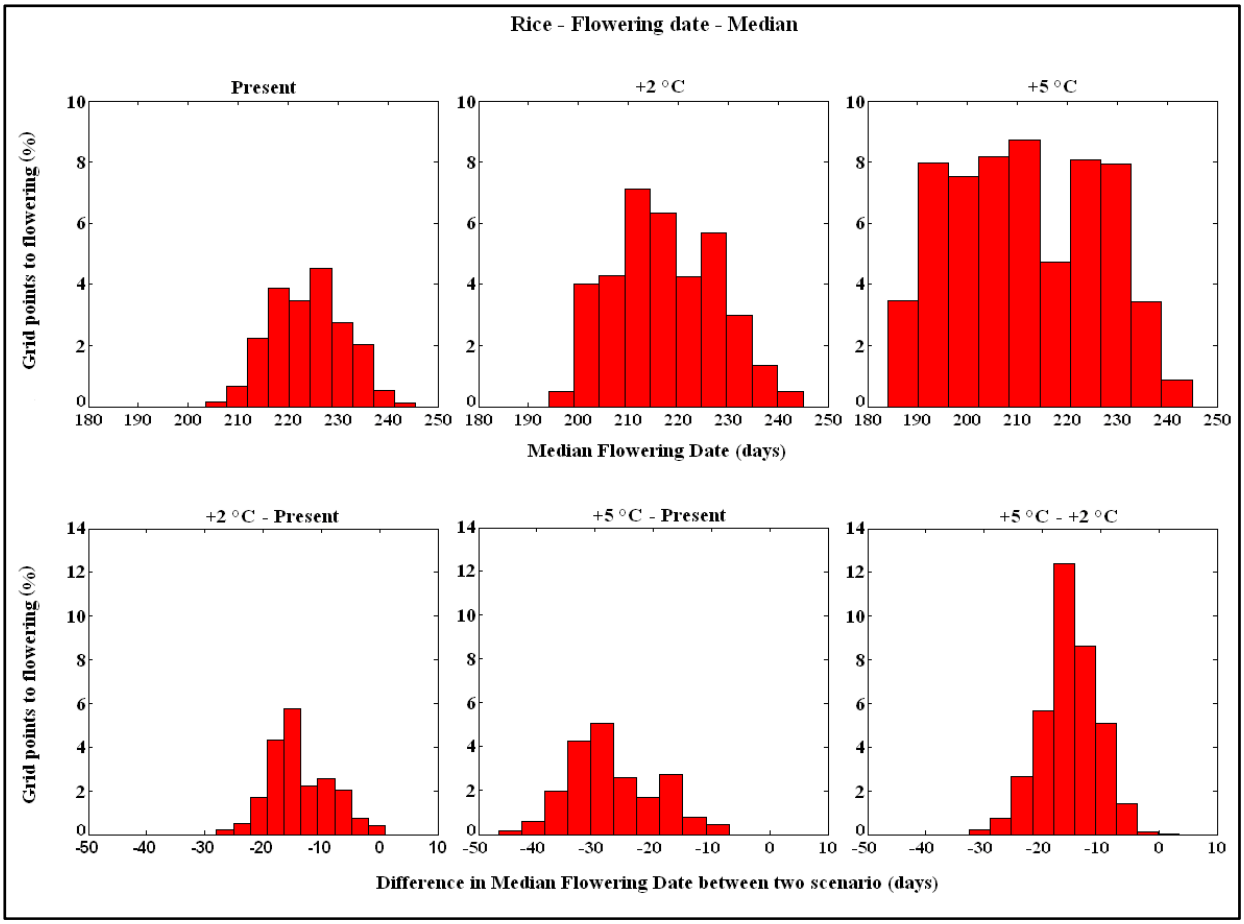

Figure 33: Distribution of median flowering dates of rice over Europe in the reference situation, the + 2 °C and + 5 °C scenarios, as well as differences between the two scenarios

The distribution of the median harvest dates of rice cultivated throughout Europe is presented in Figure 34. The median harvest date in the reference situation (present scenario) was mostly at about day 263. In the + 5 °C scenario, the median harvest date was advanced by 27 days in most grids. However, shifts in harvest dates varied widely. In the + 2 °C scenario (relative to the reference situation), a shift of 14 days was observed in most grids (Figure 34).

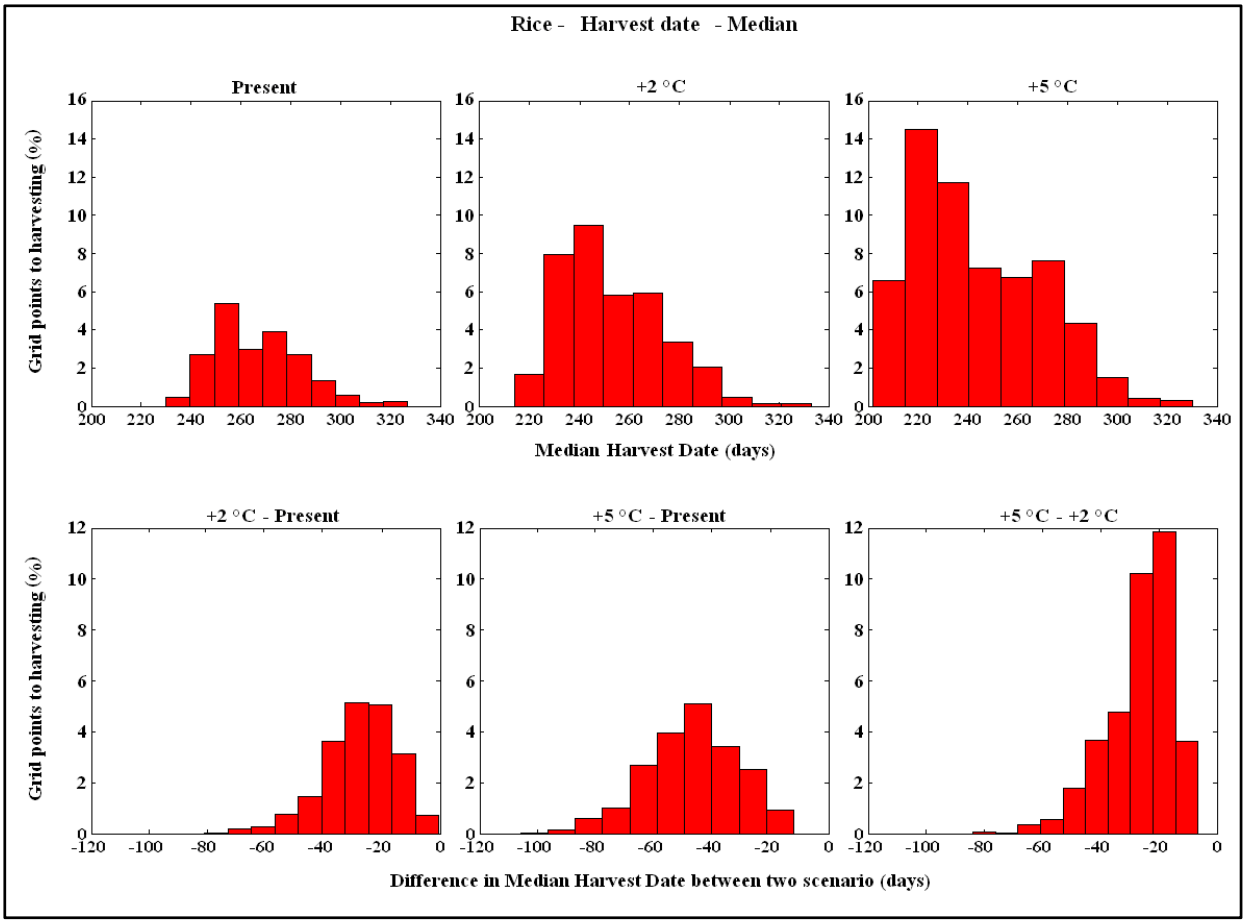

Figure 34: Distribution of median harvesting dates of rice over Europe in the reference situation, the +2°C and +5°C scenarios, as well as differences between the two scenarios

3.4. Prototype model for AFB_1 prediction at harvest

3.4.1. Development of the prototype model in maize

The rates obtained for *A. flavus* growth and AFB_1 production were linked to obtain the ***A. flavus* sub-model**. It included SPO, DISP, GER, INF, INV and AF (see 3.1 for details). They were all computed as functions of T, RH and Rain. The simulation started from flowering, with 21 days of crop susceptibility, intended as the period when silks are a suitable substrate for the fungus inoculation.

The model was developed to use hourly data as input because the functions developed from research data were hourly based.

A **crop sub-model** (maize) was also developed and linked to the *A. flavus* sub-model. In fact, crop susceptibility starts with flowering, better defined by silk emergence in maize, and the fungus continues to grow till to crop harvest, if temperature and a_w conditions are suitable.

The JRC database was used to determine the crop phenology. Seeding data were requested and maize emergence was computed by the JRC model. Seeding dates, and consequently emergence dates, can change in different areas and years for several reasons, mainly because of meteorological conditions, but also due to farmers choice. In order to standardise the simulation at the European scale, the emergence was fixed at 20 April and the maximum season length, from emergence, was fixed at 200 days.

Tsum1 was defined as the summation of DD (base 10 °C; see section 1.3.3.3 for details) from crop emergence to flowering. It was chosen at 750 DD. Tsum2 was defined as the summation of DD (base 10 °C) from crop flowering to harvest. It was chosen at 750 DD. Kernels a_w was estimated based on the function described in section 3.1.6.

The final output of the model was the **AF risk index**. The index values quantified the level of predicted risk for AFB_1 contamination at harvest.

3.4.2. Validation of the predictive model for AFB_1 in maize

A nine years data survey of AFB_1 contamination in maize inform north Italy (Battilani et al., 2008a, b, c) were considered in order to validate the model. An ANOVA was run taking into account factors such as "year", "sampling place" and the interaction between these two factors. Twenty five per cent of the variability in AFB_1 contamination detected at harvest was explained by the three factors summarising well the weather conditions. Other factors are known to play an important role, as already stated in the introduction of this report. Therefore, a limited percentage of variability can be explained by meteorological data input.

A wide variability was found in AFB_1 contamination among the samples collected in different geographic areas in the same year and among years (Figure 35). In addition, a wide variability was observed between samples collected in the same province, a small geographic area (see Figure 36 as an example).

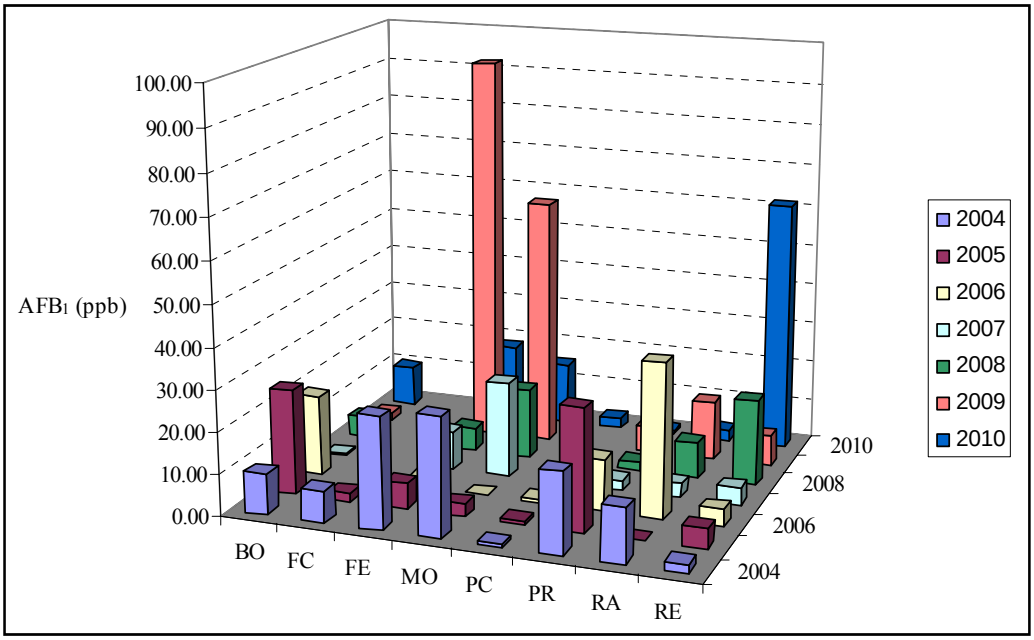

Figure 35: Mean AFB1 contamination in eight provinces (BO: Bologna – FC: Forlì Cesena – FE: Ferrara – MO: Modena – PC: Piacenza- PR: Parma – RA: Ravenna – RE: Reggio Emilia) of the Emilia Romagna region inItaly between 2004 and 2010

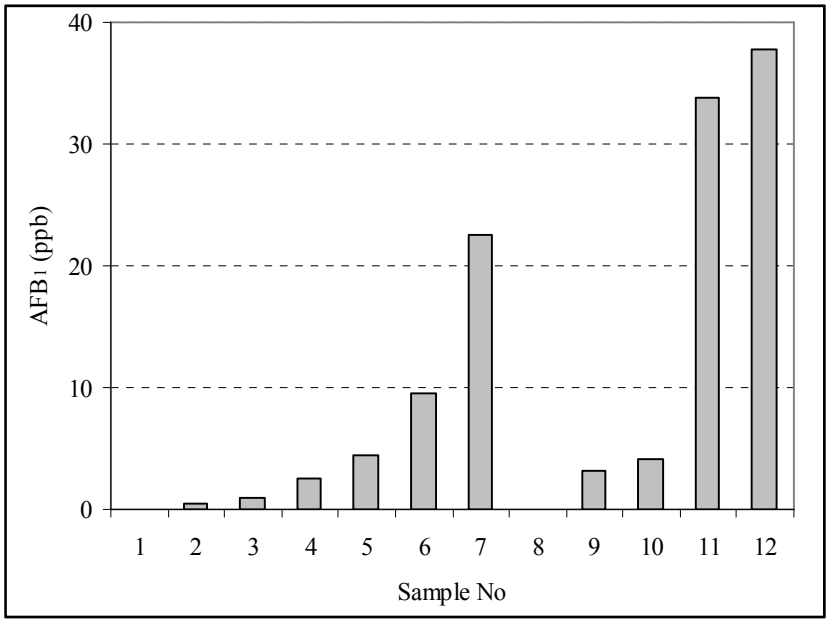

Figure 36: Aflatoxin B_1 contamination in 12 maize samples collected in Bologna province (north Italy) in 2004

Based on the above data, it is concluded that model validation can only be done at the geographic area level, while for a validation at the field level further data on cropping system would be necessary, but it is not appropriate for the aim of this project.

3.4.3. Adaptation of the maize predictive model to use daily meteorological data

The model developed to predict AFB_1 contamination at harvest in maize was run using mean daily meteorological data as input instead of hourly data for the eight provinces of the Emilia Romagna region shown in Figure 35; the data collected over a three years period (2008-2010) were considered.

AF indexes (i.e. indicators of the possible occurrence of AFs) computed with hourly and mean daily meteorological data were correlated (Figure 37). The mean meteorological data gave an overestimation of the risk, mainly with low risk. This is probably due to the loss of precision in predictions due to the lower level of definition of data input. Nevertheless, because climate change scenarios data are at daily scales and because the aim of the project is to show climate change scenarios at the European scale, the use of a model developed with mean daily data as input was considered.

AF indexes, based on daily data collected in 2008-2010 in north Italy, ranged between 0 and 140; according to the AFB_1 contamination data, AF indexes below 40 corresponded to very low/no risk of AFB_1 in maize kernels at harvest.

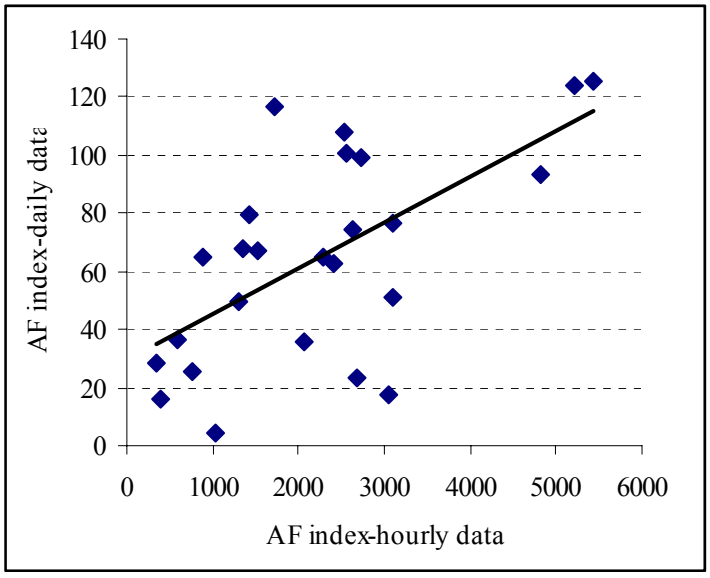

Figure 37: AF index based on hourly (x axis) and daily (y axis) meteorological data

3.5. Prototype model for AFB_1 prediction at harvest in wheat and rice

No data were found in the literature regarding AFs contamination in rice at harvest and only one paper reported wheat contamination in Algeria (Riba et al., 2010). This result suggested that AFs contamination in these crops at harvest was not an issue, irrespective of climate conditions. Nevertheless, *A. flavus* infection in wheat and rice in the field cannot be excluded because post-harvest contamination was previously signalled, mainly in rice and it is well known that fungal inoculum come from the field and can be followed by post-harvest infections.

Alerts have been reported by RASFF for AFs in basmati rice for direct human consumption imported from Pakistan (i.e. in 2008, 2009, 2010 and 2011); several highly contaminated lots were found and an increase (+50 %) of official controls at the designated point of entry have been established by the Commission Regulation 669/2009[12]. Post-harvest crop management can improve considerably the prevention of mycotoxin contamination. However, since fungal inoculum is commonly acquired in the field, agriculture production management should also be taken into consideration.

Research is necessary to understand these aspects and to define if, when and how, *A. flavus* infection is possible in wheat and rice during the growing season. Any prediction without this scientific base is purely theoretical. Nevertheless, the aim of this project was to model, predict and map the emergence of AFs in cereals in the EU due to climate change. The idea was to consider the possible effect of changes in weather conditions on the fungus in relation to the adapted behaviour of the host crops. Because of the lack of scientific information regarding the infection cycle of *A. flavus* on wheat and rice, several assumptions were made.. It was assumed (i) the infection was possible on wheat and rice ears and (ii) the period of ears susceptibility to *A. flavus* infection was "flowering", accordingly to maize and as clearly defined for other mycotoxin producing fungi, i.e. fusaria.

The ***A. flavus* sub-model** described for maize was also used for wheat and rice. In fact, the behaviour of the fungus in different ecological conditions is not influenced by the host, but obviously host-pathogen interactions are very relevant.

[12] Commission Regulation (EC) No 669/2009 of 24 July 2009 implementing Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin and amending Decision 2006/504/EC. OJ L 194, 25.7.2009, p. 11–21.

3.5.1. Development of the prototype model for wheat

The **crop sub-model - wheat** was developed with data from the JRC. In particular, the starting point for crop phenology simulation was assumed to be the 1st of January. Tsum1, intended as the DD from 1st January to flowering was considered at 800. Tsum2, the DD from flowering to harvest was considered at 970. No base was considered for DD calculation. Maximum season length, from 1st January, was fixed at 300 days.

The simulation of *A. flavus* started from flowering, with 21 days of crop susceptibility, until crop harvest.

Water activity in kernels was estimated based on the function described by Rossi et al. (2007); this function describes the dynamic of a_w as a function of days (d) elapsed from heading:

$$a_w = 1 - \exp(-27.53 * \exp(-0.07 * d))$$

This function was developed using the data collected in Italy, which were based on days rather than temperatures sum. Therefore, the function developed above for maize needs to be improved to give more precise data applicable to other geographic areas. Nevertheless, this function was considered and included in the *A. flavus* predictive model to predict the dynamic of a_w in wheat kernels.

The final output of the model was the AF index. The levels of risk for AFB_1 contamination at harvest was predicted based on the index values, as described for maize.

3.5.2. Development of the prototype model for rice

The **crop sub-model - rice** was developed based on studies published by Confalonieri et al. (2009a; 2010a; 2010b; 2009b). In particular, the starting point for rice phenology simulation was fixed at crop seeding. Similarly to the approach followed in maize, seeding time was fixed at 14 May. Crop emergence was predicted after 100 DD, flowering after 800 DD from emergence and harvest after 430 DD from flowering; the base T for DD computation was 12 °C. No maximum season length was fixed.

The simulation of *A. flavus* started from flowering, with 21 days of crop susceptibility, in agreement with the assumption applied to the other crops, untill crop harvest.

The dynamic of kernels a_w has never been considered in the literature, but one paper described its variation in time with humidity (Ishimaru et al., 2009). It was assumed that humidity and a_w follow a similar trend and, based on the published data, a function was developed to predict the dynamic of a_w as a function of days (d) elapsed from heading.

$$a_w = 13.7022 * d \text{ ^ } 0.867$$

The fitting with published data was very good ($R^2 = 0.996$).

Similar comments to those reported for wheat could be made; this function needs to be improved to give more precise data applicable to other geographic areas. Nevertheless, this function was considered and included in the *A. flavus* predictive model to predict the dynamic of a_w in rice kernels.

The final output of the model was the AF index. The level of risk for AFB_1 contamination at harvest was predicted based on the index values, as described for the two other crops.

3.6. Mapping AF risk index in the EU in different climate change scenarios

The predictive model for *A. flavus* was run for maize, wheat and rice all over Europe according to the above crop sub-models. All meteorological data were used as input, intended as actual, + 2 °C and + 5 °C scenarios for 90 time series, defined as 2011-2100.

The results were reported in maps for some selected years, according to section 3.2.1, 3.2.2 and 3.2.3 for wheat, maize and rice, respectively.

The maps were obtained using the EasyKrig 3.0 software developed by Dezhang Chu with funding from the National Science Foundation through the U.S. GLOBEC Georges Bank Project's Program Service and Data Management Office.

The maps were prepared following five steps:

(i) output data of the *A. flavus* model, and the AF risk indexes (see below) for each grid point, including grid information (Latitude, Longitude, Altitude), were prepared in ASCII format;

(ii) variograms were calculated in order to generate a data-based semi-variogram, then a model-based semi-variogram/correlogram to fit the data-based variogram just computed;

(iii) original data, computed in specific grid points (50 x 50 km grid), were interpolated and extrapolated using the Kriging method;

(iv) mapping Toolbox (Matlab), a software useful to draw maps and perform geospatial data analysis, was used for data visualization and mapping;

(v) all maps were based on the coastline and land areas provided by Matlab, based on the Mercator projection with the same colours corresponding to the same AF risk indexes.

Four different levels of occurrence were identified:

AF indexes between 141 and 180 (from red to dark red in the maps) were associated with a high AFB_1 risk;

AF indexes between 101 and 140 (yellow to orange in the maps) were associated with medium AFB_1 risk;

AF indexes between 41 and 100 (green to pale blue in the maps) were associated with low AFB_1 risk;

AF indexes below or equal to 40 (Blue to violet in the maps) were not associated with a negligible (or no) risk of AFs contamination in the field.

3.6.1. AF risk index in maize

The output of the *A. flavus* predictive model in maize(AF index) was mapped for Europe in the years 2016, 2018, 2020, 2079, 2087 and 2088; the maps for the year 2079 are given in Figures 38 to 40 and the maps for the other years are in Battilani et al. (in prep.). The year 2079 was considered with a mean meteorological condition in the period 2000-2100, with 25.6 °C as mean T and 49.9 mm of rain in the period mid June-mid July in the + 2 °C scenario (see section 3.2.2 for details).

The years 2016 and 2020 were respectively the coldest and warmest years in that period with 25.2 °C and 26.1 °C as mean T and very similar rainfall patterns. The year 2088 was added as the driest year, with 48.1 mm of rain and a T very close to the mean (i.e. 25.6 °C). The years 2018 and 2087 were

included both as wet years, with 51.3 and 51.6 mm between 15 June and 15 July, and a mean T of 26.1 °C and 25.6 °C, respectively.

In the actual meteorological scenario (Figure 38), areas which indicated a predicted risk value above zero were essentially located in the IBERIA, ITALIA and BALKGREE macroareas. These macroareas were very similar in the four years and showed an increase in the index in the hottest year, but more particularly in the driest year.

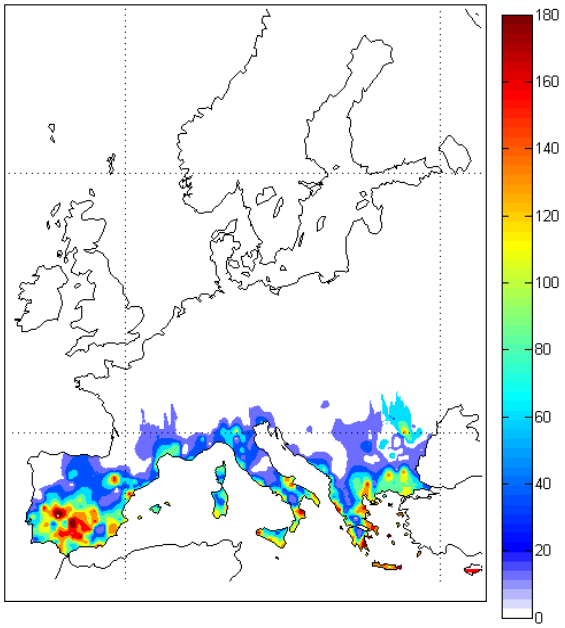

Figure 38: Map reporting the risk for aflatoxin B_1 contamination in maize in the actual meteorological scenario. The year 2079 is the mean meteorological condition in the period 2000-2100

A risk of spread of AFB_1 contamination towards northern areas in the + 5 °C climate change scenario (Figure 40), especially in the warm years 2018 and 2020 and the driest year 2088 (Table 15) was highlighted. In agreement with crop phenology predictions (see section 3.3.2), the + 5 °C scenario leads to a shorter season for maize, with earlier flowering (5-20 days earlier) and harvest (20 days in advance as median). Consequently, the growing area for maize, as well as suitable conditions for the fungus, were expected to move towards the north. Nevertheless, conditions seemed to become less favourable for AFs production and, compared to the + 2 °C scenario, to reduce the potential for a high risk in these areas.

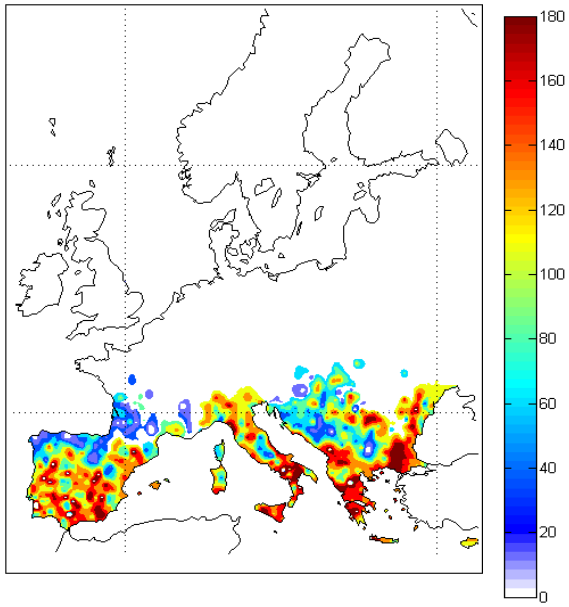

Figure 39: Map reporting the risk for aflatoxin B_1 contamination in maize in the +2 °C climate change scenario.The year 2079 is the mean meteorological condition in the period 2000-2100

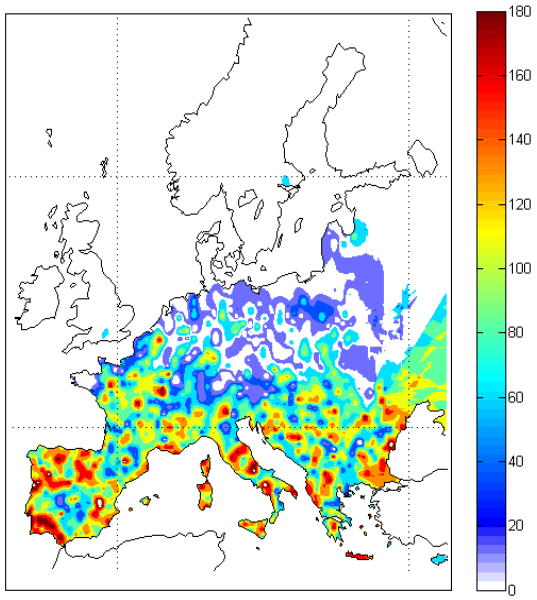

Figure 40: Map reporting the risk for aflatoxin B_1 contamination in maize in the +5 °C climate change scenario. The year 2079 is the mean meteorological condition in the period 2000-2100

3.6.2. AF risk index in wheat

The AF index in wheat was mapped for Europe in the years 2016, 2020, 2021, 2050, 2072 and 2082; the maps for the year 2050 are shown in Figures 41 to 43 and the maps for the other years are in Battilani et al. (in prep.). The year 2050 was considered with a mean meteorological condition in the period 2000-2100, with 19.4 °C as mean T and 50.5 mm of rain in the period mid June-mid July in the + 2 °C scenario (see section 3.2.1 for details).

The years 2016 and 2020 were respectively the coldest and the hottest years in that period, as reported also for maize, with 18.9 °C and 20.0 °C as mean T and very similar rainfall around 50 mm. The years 2021 and 2072 were both wet years, with temperatures above and below the mean value, respectively, for the 100 year period. The year 2082 was added as the driest year, with 48.8 mm of rain and a T (19.2 °C) very close to the mean (19.4 °C).

In the actual meteorological scenario (Figure 41), a risk value above zero was highlighted in a small area east of the EEUROPA macroarea.

Moving to the + 2 °C climate change scenario (Figure 42), the risk area was very similar, although slightly wider.

A risk of spread of AFB_1 contamination towards northern areas was highlighted in the + 5 °C climate change scenario (Figure 43), although no true risk of AFs contamination in the field (see section 3.6) was depicted in all the three scenarios (i.e. AF risk indexes were below 40).

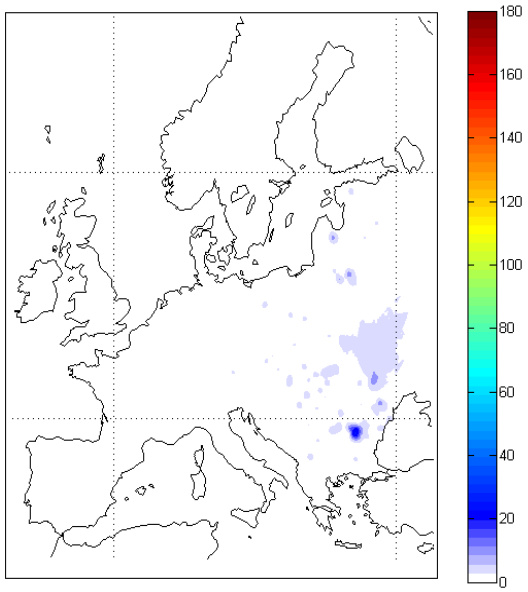

Figure 41: Map reporting the risk for aflatoxin B_1 contamination in wheat in the actual meteorological scenario. Year 2050 is the mean meteorological condition in the period 2000-2100

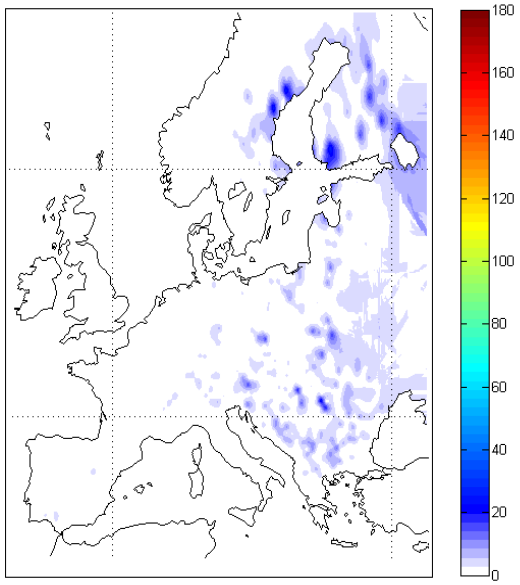

Figure 42: Map reporting the risk for aflatoxin B_1 contamination in wheat in the +2 °C climate change scenario. (Year 2050 is the mean meteorological condition in the period 2000-2100

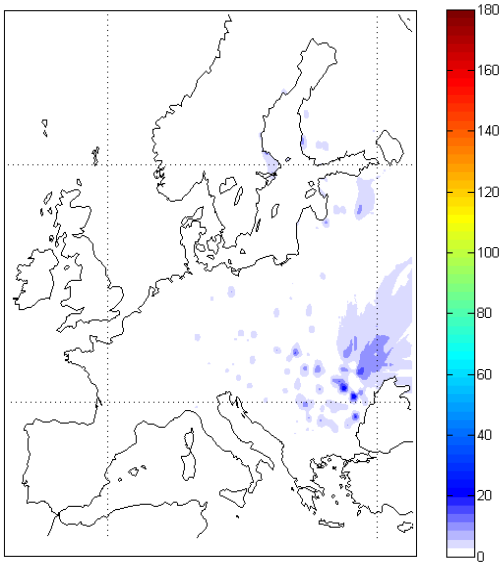

Figure 43: Map reporting the risk for aflatoxin B_1 contamination in wheat in the +5 °C climate change scenario. The year 2050 is the mean meteorological condition in the period 2000-2100

3.6.3. AF risk index in rice

The output of the *A. flavus* predictive model in rice (AF index) was also considered for rice and mapped for Europe in the years 2016, 2020, 2058, 2070, 2085 and 2099 (Figure 44). The year 2058 was considered with a mean meteorological condition in the period 2000-2100, with 26.8 °C as mean T and 43.5 mm of rain in the +2 °C scenario in August (see section 3.2.3).

The years 2016 and 2020 were respectively the coldest and warmest years in that period, with 26.4 °C and 27.5 °C as mean T and very similar rainfall around 43 mm. The years 2070 and 2099 were the most rainy, with a mean temperature very close to the mean reported for the year 2058. The year 2085 was added as the driest year, with 41.5 mm of rain and about the same T (26.9°C) as the mean (26.8 °C).

The output of the predictive model highlighted a risk index close to 0 or not computed (field conditions did not allow successful infections by *A. flavus*) in all climate change scenarios and years considered (Figure 44). This is in agreement with the literature that never signalled rice contamination with AFs at harvest.

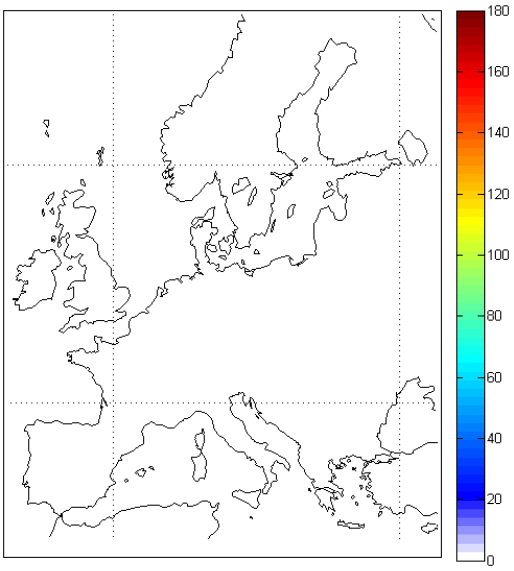

Figure 44: Map reporting the risk for aflatoxin B_1 contamination in rice in all the climate change scenarios and years considered

3.7. Sensitivity analysis of AF predictive model

The AF risk index in wheat in 2050 in the present scenario, as mean of the whole considered area, was 0.81. Variations in AF indexes, obtained with the sensitivity analysis, were reported as percentage of variation compared to the reference value.

The model showed a high sensitivity to T variations, mainly to T increases. The risk variation was not linear, as expected, because complex functions contributed to the final output of the predictive model. The relevant increase of mean AF risk index was due to two components, the increased in the number of grid points associated to an AF risk index and the increase of each point index. In other words, when T increased, *A. flavus* infection became possible in new grid points where the conditions were not suitable in the considered scenario; besides, in each grid point, the conditions became more appropriate for fungal growth and AFs synthesis and the AF risk index increased consequently.

The model was less sensitive to RH and AF risk index was negatively related to this parameter, as expected; an increase of 30 % in RH was requested to change significantly the model output and very limited variations were observed with RH decrease.

Table 21: Relationships between T, RH perturbation and model output (AF risk index) variation from the reference value (value for average condition without perturbation)

Parameter modified	Perturbation	AF risk index variation (%)*
Temperature (°C)	+ 2 °C	+ 26
	+ 4 °C	+ 84
	+ 6 °C	+ 152
	- 2 °C	- 17
	- 4 °C	- 30
	- 6 °C	- 39
Relative Humidity (%)	+ 10 %	- 2
	+ 20 %	- 8
	+ 30 %	- 27
	- 10 %	+ 0.1
	- 20 %	+ 0.25
	- 30 %	+ 0.25

(*): It was computed comparing the mean AF risk index of wheat in the whole considered area (0.81) with the risk index obtained changing T and RH; the variation was expressed in %

As a general comment, the sensitivity analysis underlined that validated meteorological data, without systematic errors, are essential to perform an accurate simulation, because significant variation in the predicted risk are generated with input data variations. The model showed a high sensitivity to T variations; because T is a parameter that is normally measured and predicted more accurately than water related parameters (like rain or RH), it is expected to provide reliable outputs from the developed model.

4. DISCUSSION

In order to assess the AF risks predicted by the selected climate change scenarios, the AF indexes must be linked with the current crop production patterns seen in the European territory. The risk for AFB_1 contamination in rice and wheat was negligible (Figures 41, 42, 43 and 44), whereas risks for AFB_1 contamination in maize appeared to be of concern.

Based on the EUROSTAT 2003 - 2009 database on Agricultural products including data on regional crop production (http://epp.eurostat.ec.europa.eu/ portal/page/portal/agriculture/data/ database), maize production in Europe was found in Romania (2,338,900 ha), France (1,679,800 ha), Hungary (1,177,300 ha), Italy (915,500 ha), Germany (464,300 ha), Spain (347,600 ha), Croatia (296,900 ha), Bulgaria (274,200 ha), Poland (274,100 ha) and Greece (240,000 ha) (Eurostat, 2009). According to the scenarios made on AF risks, countries of high interest were Spain, Italy and Greece. Other countries, such as Portugal, France, Bulgaria, and Romania also deserve attention since they showed hot spots (localized areas) of AF risk occurrence.

A number of AF risk maps were produced for maize, wheat and rice under the three selected climate change scenarios (Battilani et al., in preparation). In the actual scenario, the year 2079[13] representing the mean meteorological conditions in 100 simulated years (Figure 38), highlighted the presence of AF risk spots in three critical macroareas namely IBERIA, ITALIA and BALKGREE. The combination of a dry season, during maize flowering time (i.e. from mid-June to mid-July), with high temperatures (all values above the average value of 25.6 °C), resulted in high risk areas with AF indexes between 141 and 180 in central-south of Spain, southern regions of Italy, in south-east of Greece and in Cyprus. Out of the three macroareas, IBERIA, mainly Spain, had the largest AF risk area with an AF index above 100. This is probably due to favourable meteorological conditions for *A. flavus*, i.e. high temperatures and a significant dryness, which dramatically favour AF production during the period of maize flowering (Battilani et al., 2008b).

In Spain, the critical districts were located around Extremadura, Castilla la Mancha and Andalusia, all together representing 31 % of the overall maize production area of this country (Eurostat, 2009).

Regarding the ITALIA and BALKGREE macroareas, the areas of concern were located in small zones of the east of Calabria and west of Sicily in Italy, and in Attika, east Thessalia, west Epiro and south-east Peloponnese in Greece. Maize production in these areas equals to 0.7 % and approximately 20 % of the Italian and Greek maize production, respectively (Eurostat, 2009). Given the low level of maize production in these areas, one may think that the overall AF risk is low. However, as the whole production might be consumed entirely by the local human and animal populations, the AF risk in these areas should not be overlooked. Therefore, monitoring efforts to control AF in maize crops, in these areas, at harvest should be maintained.

A risk area comparable with the one shown in the year 2079 has been drawn in the other considered years (Battilani et al., in preparation). The years 2016, 2018 and 2087 showed slightly lower AF risk indexes in the same macroareas, which is coherent with the average values of temperature and rain obtained for the above-mentioned years.

The warmest and driest years (2020 and 2088, respectively) were found to represent a major concern, mainly in the IBERIA macroarea because it showed a larger zone with AF index above 140, with the highest risk related to the driest year (2088).

[13] Years in the context of this report do not represent the specific calendar years, but the simulated years after running the climate model. For example, conditions described for the year 2079 are the predictions of the 79th run according to the generated meteorological data (for more details see section 2.3.2).

In conclusion, for the scenario based on current climatic conditions, the EU countries where maize is widespread, such as Romania, France and Hungary having 28 %, 20 % and 14 % of the EU-27 production, respectively, showed a low risk of AF occurrence.

In the + 2 °C climate change scenario, the EU areas of interest for AF risk were extended in the EEUROPA macroarea (Figure 39). The traditional European agricultural areas such as Spain (excluding north), south of Italy, the BALKGREE macroarea and also Turkey (European Turkey only) were of concern as they were characterised by zones of AF indexes between 141-180. Moreover other countries such as Albania and east of Bulgaria entered in the panel of countries presenting high risk areas, while Romania, Hungary and France showed areas with low and medium AF indexes.

Similar to the scenario based on current climatic conditions, the risk areas drawn in 2016, 2018 and 2087 (Battilani et al., in preparation) were very similar to the one shown in the year 2079; both the extension of the risk area and the risk index were comparable. New spots, with AF risk indexes below 100, but frequently below 40, were added in the years 2020 and 2088; moreover, in the driest year 2088, higher risk indexes were highlighted in southern Spain and central-north Italy.

In the + 2 °C scenario, although there was a predicted slight advance of 5 and 10 days for flowering and harvesting dates (Figures 29, 30), respectively, the AF risk index was not expected to change because the agricultural management practices were not expected to be modified neither. However, the enlargement of AF risk zones highlighted the potential increase of human and animal population chronic exposures. Therefore strategies on the development of specific crop management are recommended.

In the + 5 °C scenario, the increase in temperature depicted a different situation for the year 2079. A considerable enlargement of the geographical area which had an AF index above zero (Figure 40) and a reduction of the geographical area with AF risk indexes between 140-180 were found. The enlargement in central Europe included AF indexes below 80. The extended area in south-east Europe represented medium to low AF risks, whereas a large portion of the central-north of Europe represented a low risk. Finally, almost no risk was anticipated in the ENG and SCAN macroareas.

The variability between years was more significant in the +5 °C scenario than in the other two scenarios considered. In 2016 and 2087, an AF risk was predicted in a smaller area as compared to the average reference year (2079). In the year 2018, the risk was estimated to cover an area comparable in size, but with a different geographical pattern, as compared to 2079. The most remarkable change was drawn for 2020, the hottest year, with a spot of medium risk also predicted in the SCAN macroarea, while in 2088, the driest year, the medium-high risk areas were comparable with 2079.

It should be noted that, in this + 5 °C scenario, a significant advance in flowering and harvesting dates was estimated (10 and 15-20 days, respectively; Figures 29 and 30) implying possible changes in the maize susceptibility to fungal attacks and subsequent AF production in the field. Moreover, the potential for high AF concentrations in grain decreased with increasing number of rainy days and days with a relative humidity above 70 % and with temperatures above or below the optimum for fungal growth. The + 5 °C scenario led to a larger European territory exposed to the risk of AF contamination. However, despite this figure, the increase in AF indexes in southern Europe was not of concern since conditions were less suitable for *A. flavus* growth.

Good agricultural practices for the mitigation of AF contamination in maize are not standardised since AFs are still not considered an issue in EU at pre-harvest (i.e. during growth in the field). They are predominantly seen as a problem in tropical areas at post-harvest (i.e. during storage).

It was not yet possible to link the AF risk index with the probability of exceeding the legal maximum levels of AF contamination established by EU for food and feed (Regulation EU/1881/2006 and amendments and Commission Directive 2003/100/EC)[7], but it was clear that both in the + 2 °C and + 5 °C climate change scenarios, the risk for AF contamination in maize increased in Europe with a different pattern throughout the area to that existing today.

Even if the extent of AF exposure was not quantified, the exposure to AFs appeared to be of concern, given the higher percentage of human and animal populations exposed to low levels of Afs, and long-term exposures in EU.

For all three climate change scenarios (current, +2°C and +5°C), different figures were drawn for wheat and rice. Wheat presented an increase in the AF risk index in some areas, but always amenable to very low risk levels, whereas rice showed no risk. Nevertheless, post-harvest contamination is signalled in both products and the role of the pre-harvest conditions in supporting fungal inoculums still need to be clarified.

In this context, the implementation of official European and National control plans would help minimizing the exposure of both animal and human populations to AF risks, while specific control measures should be identified and adapted to the areas belonging to the different risk levels. This approach should be extended to those cases in which the maize is produced for self-consumption and national controls are not applied.

5. CONCLUSIONS AND RECOMMENDATIONS

KNOWLEDGE GAPS

The literature search conducted in this study highlighted knowledge gaps, both on host plant-pathogen interactions and the occurrence of AFs, in particular for AFB_2, AFG_1 and AFG_2, for most of the European territory and for wheat and rice crops. To fill some of the gaps and develop predictive models, the consortium conducted some research to collect missing data on *A. flavus*. Nevertheless, to further test and improve the models, the collection of more data in the following areas are recommended:

The role of water activity (a_w) is described as a crucial parameter in all studies dealing with *A. flavus* and *A. parasiticus* growth. The effect of a_w has been quantified in *in vitro* trials, essentially on artificial media, but no data was found on the effect of a_w in *in vivo* conditions. However, it is reasonable to expect that the effect of a_w varies in vivo because field observations showed that AF production is enhanced when kernel humidity is below 32 %, which is not supported by *in vitro* trials. Data collection to understand the role of a_w in fungal growth and AFs synthesis in maize ears is not straightforward, but crucial for improving the predictive model, in particular the mycotoxin production rate.

The dynamic of a_w in kernels has been studied accurately in maize but this information is missing in wheat and rice. Sampling of kernals during the growing season and/or at harvest would allow the collection of useful data to improve and to validate the predictions made on wheat and rice and confirm the predicted low/absent AF risk in these crops.

The model has been validated on maize with data only coming from surveys conducted in Italy because georeferenced data and related meteorological data on AFs contamination of maize at harvest were not available for other geographic areas. In order to produce a more robust model, the collection of more such data from other EU countries is recommended.

AFLATOXINS RISK INDEXES

Taking into account the three selected climate change scenarios (i.e. current, +2 °C and +5 °C), the levels of production of the three crops (i.e. maize, wheat and rice) and their associated AF indexes, the following AF risks were identified and evaluated:

Critical and high AF risk indexes corresponding to high aflatoxin risks were highlighted in some areas of southern European countries (i.e. in Greece, southern Italy, Bulgaria, Albania) under the climate change scenario that is the most favourable to *A. flavus* (+2°C scenario). Although these countries do not belong to the main European maize producers, it has to be considered that the locally produced maize might go directly to local consumers exposing both human and animal populations to a continuous AF risk. Fungal infections in the field may be an important source of mycotoxin production which may occur and increase in the subsequent supply chain stages. Fungi can be active during the post-harvest period and AF contamination may increase dramatically if the drying and storage phases are poorly managed. Therefore, the highlighted predicted risks could be used by risk managers to take proper control measures to mitigate AF risks.

High AF index corresponding to high AF risks were shown in the south of EU (i.e. in central and southern Spain, north-east Greece and Cyprus) in the three climate change scenarios, where production of the three crops are high (e.g. Spain). Overall, the increase in AF indexes led to higher risk situations, but it is not possible to quantify and compare these levels to the legal maximal levels. It is recommended to identify good agricultural practices and post-harvest managing measures favouring safe production and prevention from potential AF contamination. Integrated control management measures have to be implemented to improve not only agricultural practices, but also the entire food and feed chain management, including storage where the AF contamination can increase significantly.

Low and medium AF risk indexes corresponding to low and medium AF risks were found in the four main maize production countries (i.e. in Romania, France, Hungary and north-east Italy, all accounting for 73 % of the total of EU-27 production in 2009), in the most favourable climate change scenario for *A. flavus* growth (+2 °C). Currently, due to EU legislations, EU Member States, within their annual national control plans, should already have implemented measures to monitor AF contamination, and therefore have put in place an effective warning strategy for mycotoxin control.

Low AF index corresponding to low aflatoxin risk was highlighted in the northern European countries. These countries are currently in the safeguard zone regarding aflatoxin risk due to their climatic conditions, but if the +5 °C scenario is considered, they will face new climatic conditions leading to a new agro-socioeconomic context. Because of the low AF index, it is anticipated that with appropriate control measures, the risk can be restrained. Even if the predicted conditions are in favour of a spread of AF contamination, the monitoring of the fungal and mycotoxin occurrence will confirm or discount the scenario of new fungal niches arising from the changed climatic conditions.

Very low AF indexes corresponding to very low AF risks were detected in all European countries for wheat and rice crops. This reflects what is already documented in the literature. However, these two crops could be susceptible to more fungal infection during post-harvesting (e.g. during storage), probably due to the infection of kernels in the field. In the case of rice, based on the information provided by the RASFF on AF contamination, there is a need to ascertain if the contamination originates from storage or from the field.

From a risk assessment perspective, the AF risk maps produced in this report could be used as a communication tool for stakeholders, especially for farmers and stockbreeders, to catch their attention on the issue of AF contamination and to highlight to them the importance of mycotoxins within the food safety control in the food and feed chain. The risk maps could also be used as a management tool to enhance the evaluation of the risk of mycotoxin contamination and to prioritize mycotoxins within

risk assessment interventions. Maize is the crop of concern at least in some regions of the EU territory (i.e. in specific areas of Spain, Greece, Italy, Bulgaria, Albania and Cyprus), whereas wheat and more particularly rice are not at risk.

Based on the above evaluation on AF risks, detailed surveys are recommended, with different focuses for maize, wheat and rice, to produce data on AF occurrence in critical countries (e.g. in Greece, south of Italy, Albania and Bulgaria). Also, the need for collecting fit-for-purpose occurrence data aimed at evidencing possible trends of emerging risk indicators (e.g. monitoring *Aspergillus* presence in northern EU) is highlighted.

Aflatoxins are a priority issue within the EU food and feed safety policy, with the first regulation setting maximum limits for mycotoxins in foodstuffs adopted in 2001 (EC, 2001) and a directive setting maximum limits of AFB_1 in animal feedingstuffs adopted in 2002 (EC, 2002). In general, official control measures contribute to the global effort for AF risk reduction. However, specific action plans also need to be directed to small local farmers who are exempt from food and feed safety official controls.

Finally, it should be noted that the figures of the distribution of EU crop-harvesting are likely to evolve with climate change. For example, throughout Europe, there will be an increase in a favourable area for grain maize production and this crop, that is currently and mostly grown in southern Europe, may become viable further north or at higher-altitude areas in the south of EU.

In this new agricultural context, mycotoxin risk assessment should include a wider concept of risk evaluation, including emerging risks since new mycotoxins could arise for new fungus and plant associations making the occurrence of new mycotoxins or mycotoxins not yet considered as a new potential human and animal health threat (Tirado et al., 2010).

APPENDICES

A. LITERATURE RESEARCH ON *ASPERGILLUS* SPP. IN WHEAT

Objective of the review question	Details and results of the searches				
Aspergillus . infection in wheat	***Web of Science***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 14 June 2011		**Date of the search: 14 June 2011**		
	Date span of the search: 1945-2011		**Date span of the search:1960-2011**		
	For bibliographic databases, date of the latest database update included in the search: 1 June 2011		**For bibliographic databases, date of the latest database update included in the search: 1 June 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	Aspergillus flavus AND wheat	177	[*Aspergillus flavus* AND wheat]	175	
	Aspergillus parasiticus AND wheat	66	[*Aspergillus parasiticus* AND wheat]	59	
	Aflatoxin* AND wheat	105	[Aflatoxin*] AND [wheat]	94	
	Total number of records after removing duplicates	274	**Total number of records after removing duplicates**	255	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**274**
				Number of records excluded from the review	**255**
				Number of records included in the review	**16**

B. LITERATURE RESEARCH ON CROP GROWTH MODELS FOR WHEAT

Objective of the review question	Details and results of the searches				
Crop growth models for wheat	***Web of Science***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 14 June 2011		**Date of the search: 14 June 2011**		
	Date span of the search: 1945-2011		**Date span of the search:1960-2011**		
	For bibliographic databases, date of the latest database update included in the search: 14 June 2011		**For bibliographic databases, date of the latest database update included in the search: 14 June 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	Wheat AND model AND crop growth	1266	Wheat and model and crop growth	1111	
	Wheat AND flowering	1751	Wheat and flowering	1134	
	Wheat AND climate	2274	Wheat and climate	2061	
	Total number of records after removing duplicates	4822	**Total number of records after removing duplicates**	3816	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**5218**
				Number of records excluded from the review	**5197**
				Number of records included in the review	**21**

C. LITERATURE RESEARCH ON CROP GROWTH MODELS FOR MAIZE

Objective of the review question	Details and results of the searches				
Crop growth models for maize	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 4 May 2011		**Date of the search: 4 May 2011**		
	Date span of the search: 1984-2011		**Date span of the search: 1984-2011**		
	For bibliographic database, date of the latest update included in the search: 4 May 2011		**For bibliographic database, date of the latest update included in the search: 4 May 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	[Maize OR corn] AND model AND [growth or phenol*]	1899	[Maize OR corn] AND model AND [growth or phenol*]	1946	
	[Maize OR corn] AND flowering	2726	[Maize OR corn] AND flowering	882	
	[Maize OR corn] AND climate	6212	[Maize OR corn] AND climate	1250	
	Total number of records after removing duplicates	7571	**Total number of records after removing duplicates**	3833	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**7571**
				Number of records excluded from the review	**7531**
				Number of records included in the review	**40**

D. LITERATURE RESEARCH ON MODELLING *A.* SECTION *FLAVI* IN CEREALS CROP

Objective of the review question	Details and results of the searches				
Modelling *A.* section *flavi* in cereals crop	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 4 May 2011		**Date of the search: 4 May 2011**		
	Date span of the search: 1973-2010		**Date span of the search: 1973-2010**		
	Date of the latest update included in the search: 4 May 2011		**Date of the latest update included in the search: 4 May 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	1. *Aspergillus flavus* OR *A. parasiticus* or aflatoxin*	20137	1. *Aspergillus flavus* OR *A. parasiticus* or aflatoxin*	3691	
	2. Prediction OR model*	640147	2. Prediction OR model*	2614224	
	1 and 2	1183	1 and 2	159	
	Total number of records after removing duplicates	623112	**Total number of records after removing duplicates**	2604903	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**556**
				Number of records excluded from the review	**533**
				Number of records included in the review	**23**

E. LITERATURE SEARCH FOR CLIMATE CHANGE

Objective of the review question	Details and results of the searches				
Climate change	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 26 July 2011		**Date of the search: 26 July 2011**		
	Date span of the search: 1984-2011		**Date span of the search: 1984-2011**		
	For bibliographic databases, date of the latest update included in the search: 7 March 2011		**For bibliographic databases, date of the latest update included in the search: 7 March 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	Climate change	34472	Climate change	31251	
	Stochastic weather generator	89	Stochastic weather generator	248	
	Evaporation trends	16	Evaporation trends	1830	
	Total number of records after removing duplicates	34372	**Total number of records after removing duplicates**	30874	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**34472**
				Number of records excluded from the review	**30874**
				Number of records included in the review	**17**

F. LITERATURE RESEARCH FOR AFLATOXIN OCCURRENCE

Objective of the review question	Details and results of the searches				
Aflatoxin occurrence	***OVIDSP: CAB Abstracts (1973-2011)*** ***Analytical abstracts (1980-2011)*** ***Food Science and Technology Abstracts (1969-2011)***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 05 May 2011		**Date of the search: : 05 May 2011**		
	Date span of the search: 1969-2011		**Date span of the search:1960-2011**		
	For bibliographic databases, date of the latest database update included in the search: May 2011 week 1		**For bibliographic databases, date of the latest database update included in the search: 05 May 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	[Aflatoxin AND maize] OR [aflatoxin AND corn]	3095	[Aflatoxin AND maize] OR [aflatoxin AND corn]	1360	
	Aflatoxin AND wheat	746	Aflatoxin and wheat	298	
	Aflatoxin AND rice	776	Aflatoxin and rice	303	
	Total number of records after removing duplicates	4522	**Total number of records after removing duplicates**	1961	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**4522**
				Number of records excluded from the review	**4467**
				Number of records included in the review	**55**

G. LITERATURE RESEARCH FOR *ASPERGILLUS* SPP. IN MAIZE

Objective of the review question	Details and results of the searches				
***Aspergillus* spp in maize**	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 4 May 2011		**Date of the search: 4 May 2011**		
	Date span of the search: 1973-2010		**Date span of the search: 1973-2011**		
	Date of the latest update included in the search: 4 May 2011		**Date of the latest update included in the search: 4 May 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	maize **OR** corn	227443	maize **OR** corn	90661	
	A. flavus **OR** *A. parasiticus*	9268	*A. flavus* **OR** *A. parasiticus*	1400	
	A. flavus **OR** *A. parasiticus* **or** aflatoxin	20137	*A. flavus* **OR** *A. parasiticus* **or** aflatoxin	3262	
	(maize **or** corn) **AND** (*A. flavus* **or** *A. parasiticus* **or** aflatoxin) **AND** soil	166	(maize **or** corn) **AND** (*A. flavus* **or** *A. parasiticus* **or** aflatoxin) **AND** soil	54	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** hybrid	100	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** hybrid	76	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* ***or*** *aflatoxin*) **AND** seeding	4	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* ***or*** *aflatoxin*) **AND** seeding	1	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** seed coating	0	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** seed coating	0	
	Total number of records after removing duplicates	2705	**Total number of records after removing duplicates**	2567	
	Total (potentially eligible) number of records retrieved, excluding duplicates				**2375**
	Number of records excluded from the review				**2327**
	Number of records included in the review				**48**

H. LITERATURE RESEARCH FOR CROP MANAGEMENT FOR MAIZE

Objective of the review question	Details and results of the searches				
Crop management for maize	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 4 May 2011		**Date of the search: 4 May 2011**		
	Date span of the search: 1973-2010		**Date span of the search: 1973-2010**		
	Date of the latest update included in the search: 4 May 2011		**Date of the latest update included in the search: 4 May 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrived**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **and** pest	177	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **and** pest	47	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** pest control	33	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** pest control	34	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** irrigation	27	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** irrigation	11	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** weed control	8	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** weed control	3	
	(maize **OR** corn) **AND** (*A.flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** (manuring **OR** fertil*)	42	(maize **OR** corn) **AND** (*A.flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** (manuring **OR** fertil*)	13	
	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** harvest	222	(maize **OR** corn) **AND** (*A. flavus* **OR** *A. parasiticus* **OR** aflatoxin) **AND** harvest	81	
	Total number of records after removing duplicates	169	**Total number of records after removing duplicates**	43	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**161**
				Number of records excluded from the review	**140**
				Number of records included in the review	**21**

I. LITERATURE RESEARCH FOR *ASPERGILLUS* SPP. IN RICE

Objective of the review question	Details and results of the searches				
***Aspergillus* spp infection in rice**	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 26 July 2011		**Date of the search:26 July 2011**		
	Date span of the search: 1990-2011		**Date span of the search:1990-2011**		
	Date of the latest database update included in the search: 3 March 2011		**Date of the latest database update included in the search: 26 July 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**N. records retrieved**	**Search strategies used for this data requirement**	**N. records retrieved**	
	Aspergillus flavus **OR** *Aspergillus parasiticus*	6632	*Aspergillus flavus* **OR** *Aspergillus parasiticus*	6664	
	Rice	109054	Rice	110417	
	(Aspergillus flavus ***OR*** *Aspergillus parasiticus)* **AND** rice	277	*(Aspergillus flavus* ***OR*** *Aspergillus parasiticus)* **AND** rice	262	
	(Aspergillus flavus ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** interaction	8	*(Aspergillus flavus* ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** interaction	7	
	(Aspergillus flavus ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** contamination	142	*(Aspergillus flavus* ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** contamination	136	
	(Aspergillus flavus ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** aflatoxin*	140	*(Aspergillus flavus* ***OR*** *Aspergillus parasiticus)* **AND** rice **AND** aflatoxin*	141	
	Total number of records after removing duplicates	267	**Total number of records after removing duplicates**	257	
	Total (potentially eligible) number of records retrieved, excluding duplicates				**275**
	Number of records excluded from the review				**235**
	Number of records included in the review				**40**

J. LITERATURE RESEARCH FOR CROP GROWTH MODELS FOR RICE

Objective of the review question	Details and results of the searches				
Crop growth models for rice	***OvidSP-CAB Abstracts***		***Scopus***		
	Justification for choosing the source: scientific relevance		**Justification for choosing the source: scientific relevance**		
	Date of the search: 26 June 2011		**Date of the search: 26 July 2011**		
	Date span of the search: 1990-2011		**Date span of the search: 1990-2011**		
	For bibliographic databases, date of the latest database update included in the search: 26 June 2011		**For bibliographic databases, date of the latest database update included in the search: 26 June 2011**		
	Languages included: All		**Languages included:** All		
	Search strategies used for this data requirement	**Number of records retrieved**	**Search strategies used for this data requirement**	**Number of records retrieved**	
	Rice **AND** growth stages	1551	Rice **AND** growth stages	1867	
	Rice **AND** phonological stages	24	Rice **AND** phonological stages	39	
	(model **OR** modelling) and rice	5479	(model **OR** modelling) and rice	5412	
	Rice **AND** climate change	504	Rice **AND** climate change	315	
	Crop model	579	Crop model	16316	
	Rice **AND** distribution	6095	Rice **AND** distribution	3346	
	Rice growing area	163	Rice growing area	691	
	Total number of records after removing duplicates	1535	**Total number of records after removing duplicates**	6015	
				Total (potentially eligible) number of records retrieved, excluding duplicates	**7512**
				Number of records excluded from the review	**7482**
				Number of records included in the review	**30**

K. ENDNOTE LIBRARY-LITERATURE

All citation find applying the key words (see Appendix A-J) have been saved in an EndNote file named FULL-EN_Final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

L. ENDNOTE LIBRARY-CITATIONS

The selected citations, all cited in the report, are saved in SEL_EN_Final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

M. QUANTITATIVE DATA ON *ASPERGILLUS* INFECTION CYCLE-SPORULATION

All quantitative data available in literature regarding *A. flavus* and *A. parasiticus* **sporulation** have been included in an Excel file. The file has one or more sheets, each named with the first author of the paper where data have been published and the number of the citation in SEL_EN_final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

N. QUANTITATIVE DATA ON *ASPERGILLUS* INFECTION CYCLE-DISPERSAL

All quantitative data available in literature regarding *A. flavus* and *A. parasiticus* **dispersal** have been included in an Excel file. The file has one or more sheets, each named with the first author of the paper where data have been published and the number of the citation in SEL_EN_final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

O. QUANTITATIVE DATA ON *ASPERGILLUS* INFECTION CYCLE-GERMINATION

All quantitative data available in literature regarding *A. flavus* and *A. parasiticus* **germination** have been included in an Excel file. The file has one or more sheets, each named with the first author of the paper where data have been published and the number of the citation in SEL_EN_final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

P. QUANTITATIVE DATA ON *ASPERGILLUS* INFECTION CYCLE-GROWTH

All quantitative data available in literature regarding *A. flavus* and *A. parasiticus* **growth** have been included in an Excel file. The file has one or more sheets, each named with the first author of the paper where data have been published and the number of the citation in SEL_EN_final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

Q. QUANTITATIVE DATA ON *ASPERGILLUS* INFECTION CYCLE-AFLA PRODUCTION

All quantitative data available in literature regarding *A. flavus* and *A. parasiticus* **afla production** have been included in an Excel file. The file has one or more sheets, each named with the first author of the paper where data have been published and the number of the citation in SEL_EN_final report.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA.

R. METEOROLOGICAL DATA

Daily weather data have been produced for three different scenarios, present, + 2 °C and + 5 °C, organised in three folders, with 2254 grid point each. Meteorological data have been stored in ASCII files, one for each grid point, with a file name that identifies the grid point and the scenario, as follows:

w_ID_BaseWG, for present scenario

w2_ID_anomalyWG, for scenario + 2 °C

w5_ID_anomalyWG, for scenario + 5 °C

Each file has eight columns, as described below:

(1) Year – the 100 considered years; (2) Julian day – every day of the considered period is expressed in Julian day, the integer part of the Julian date; (3) T_{min} (°C) – the minimum daily temperature; (4) T_{max} (°C) – the maximum daily temperature; (5) Rain (mm) – the daily millimeters of rain; (6) Global radiation (MJ/m^2) - the global daily solar radiation; (7) RH (%) – the relative daily humidity; (8) T_{med} (°C) - the mean daily temperature.

A list of all grid points with their ID, geographical coordinates (Lat/Log decimal degree, WGS84) and altitude (above sea level) has been prepared.

These data are available on offline media (DVD) and can be distributed by sending a specific request to the emerging risks unit of EFSA

GLOSSARY/ABBREVIATIONS

λ	Lag phases
°Cd	Degree Celsius decade
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
AFRCWHEAT	Agricultural and Food Research Council Wheat Model
AFs	Aflatoxins
AGB	Above Ground Biomass
AI	Aridity Index
APSIM	Agricultural Production System Simulator
a_w	Water activity
BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemical industry
BCA	Biological Gontrol Agent
Bt	Bacillus turingensis
BVP	Basic Vegetative Phase
ECB	European Corn Borer
CCCma	Canadian Centre for Climate Modeling and Analysis
CCSR/NIES	Centre for Climate System Research/National Institute for Environmental studies
CERES-Maize	Crop Environment Research Synthesis - maize
CERES-Rice	Crop Environment Research Synthesis - rice
CERES-Wheat	Crop Environment Research Synthesis - wheat
CERES/DSSAT	Crop Environment Research Synthesis/Decision Support System for Agrotechnology Transfer

CFU	Colony forming units
CGMS	Crop Growth Monitoring System
CO_2	Carbon dioxide
CPA	Cyclopiazonic acid
CRISP	Crayfish and Rice Integrated System of Production
CSIRO	Commonwealth Scientific and Research Organization
CZ	Czapeck agar
d	Day
DD	Degree days
DISP	Dispersal rate
DL	Day-length
DNA	Deoxyribonucleic acid
EC	European Commission
ECB	European Corn Borer
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FD	Flowering date
g	Grams
GAP	Good Agricultural Practice
GCM	Global Climate Model
GERM	Germination rate
GFDL	Geophysical Fluid Dynamics Laboratory
GFP	Grain-Filling Phase
GR	Growth rates
GS	Growth stages

h	Canopy height
H_{max}	Maximum panicle height
ha	Hectare
HD	Harvest date
HU	Heat Unit
IARC	International Agency for Research on Cancer
INF	Infection rate
INFOCROP	Information Crop
INV	Invasion rate
IPCC	Intergovernmental Panel on Climate Change
JRC	Joint Research Centre of the European Commission
JRC/MARS	Joint Research Centre MARS database
k	Extinction coefficient for solar radiation
kDa	Kilo Dalton
kg	kilograms
km	kilometers
LA	Specific leaf area
LA_{ini}	Specific leaf area at emergency
LAT_{ill}	Specific leaf area at tillering
LAI	Leaf area index
LAI_{ini}	Leaf area index at emergency
LARS WG	LARS Weather Generator
LOD	Limit of detection
LOQ	Limit of quantification
m	meter
MACROS	Modules of an Annual CROp Simulator

MC	Moisture content
mg	Milligrams
MJ	Mega joule
ml	Milliliters
mm	Millimeters
MOPP	Optimum photoperiod
MPa	Mega Pascal
MPIM	Max-Planck-Instiut fur Matematik
Mycotox	Mycotoxin production
N	Nitrogen
NCAR	National Centre for Atmospheric Research
NCAR PCM	National Centre for Atmospheric Research Parallel Climate Model
ng	Nanograms
nm	Nanometers
ORYZA 1	Oryza sativa model 1
ORYZA 2000	Oryza sativa model 2000
PDA	Potato dextrose agar
PDT	Physiological Development Time
PFP	Panicle Formation Phase
ppb	Part per billion
ppm	Part per million
Ps	Phenological development
PSP	Photoperiod Sensitive Phase
R	Rain
RASFF	Rapid Alert System for Food and Feed
RH	Relative Humidity

RICEMOD	Rice Model
RICESYS	Rice System
Rip_{LO}	Partition coefficient to leaf at early stages
RRH	Rain/humidity factor
RUE	Radiation Use Efficiency
SPOR	Sporulation rate
SRES	Special Report on Emissions Scenarios
SUCROS	Simple and Universal CROp growth Simulator
T	Temperature
T_{base}	Temperature Base
T_{max}	Temperature maximum
T_{min}	Temperature minimum
T_{opt}	Optimum temperature for growth
TRIS	Temperature for Rice Simulations
TUE	Transpiration Use Efficiency
UV	Ultraviolet
VPD	Vapour Pressure Deficit
WARM	Water Accounting Rice Model
WE	Wang & Engel
WOFOST	WOrld FOod STudies
µg	Micrograms

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