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STRATEGIE INTEGRATE PER AFFRONTARE LE SFIDE CLIMATICHE E AGRONOMICHE NELLA GESTIONE DEI SISTEMI AGROALIMENTARI

INTEGRATED STRATEGIES FOR AGRO-ECOSYSTEM MANAGEMENT TO ADDRESS CLIMATE CHANGE CHALLENGES

MILANO
12 - 14 SETTEMBRE 2017

A CURA DI
FRANCESCA VENTURA
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DIPARTIMENTO DI SCIENZE AGRARIE - UNIVERSITÀ DI BOLOGNA

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CEREAL QUALITY NETWORK PROJECT PLUS - RQC-MAIZE: AIMS, RESULTS AND FUTURE PERSPECTIVES.
PROGETTO RETE QUALITÀ CEREALI PLUS - RQC-MAIS: SCOPI, RISULTATI E PROSPETTIVE FUTURE.

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Abstract
Evaluation of maize quality is the main aim of the three year (2014-2017) "Cereal Quality Network Project Plus - RQC-Maize" financed by MiPAAF (Ministero delle Politiche Agricole Alimentari e Forestali) in order to develop a national plan devoted to maize crop chain safety improvement and subsequent increased feed and food industry competitiveness. Maize (*Zea mays* L.), a major crop in Italy for animal feed and direct human consumption, can be infected by several fungal species, some of them mycotoxin producers (*Fusarium verticilliodes; Fusarium graminearum; Aspergillus flavus; Fusarium proliferatum; Fusarium subglutinans)*. One of the RQC-Maize Project focus is to highlight that a systematic network to monitor the occurrence and levels of mycotoxin content in maize grain represents a fundamental tool for collecting information useful to deepen knowledge about Emerging Mycotoxins (EM) and to predict annual risk exposure in Italy.
Activities performed by the Research Groups involved in the present Project, were coordinated by CREA-CI Bergamo (WP0) and organized in three Work Packages (WPs).
A synthesis of main activities performed in the Project, some outputs and future perspectives are illustrated.

Keywords: *Zea mays* L.; quality; mycotoxins; monitoring; agronomic factors; predictive models
Parole chiave: *Zea mays* L.; qualità; micotossine; monitoraggio; fattori agronomici; modelli previsionali

Introduction
Mycotoxin contamination of maize (*Zea mays* L.) grain is a worldwide threat to safety both for human food and animal feed (Balazs and Schepers 2007). Mycotoxins are secondary metabolites produced by fungi, which may be toxic to or have other debilitating effects on living organisms (Castegnaro and McGregor 1998). Regulations for the maximum mycotoxin content accepted in food and feed have been put in place in most countries; the more recent binding EU regulations on toxin contamination for human consumption and recommendations for animal feeding (European Commission, 2006a, 2006b, 2007, 2011), have forced a renewed interest in breeding efforts for ear rot resistance (Balconi et al., 2010, 2014) and for various methods of control and monitoring (Berardo et al., 2011). Good agriculture and management practices are recommended during pre- and post-harvest stages of production and processing of crops to avoid mycotoxin contamination exceeding the legal limits (Reyneri et al., 2015).
A sustainable approach can be based on the prediction of mycotoxin risk to optimize crop chain management and analytical efforts. The most influential risk factors with regard to *Fusarium* and *Aspergillus* ear rot and mycotoxin accumulation are temperature, drought stress, insect damage, other fungal diseases, and maize genotype (Miller 2001). Mechanistic models are available to predict *Fusarium verticillioides* and fumonisin, so as for *Aspergillus flavus* and aflatoxin, growth and toxin production (Battilani et al., 2003, 2014). Genotype by environment interactions are likely to be very important in determining mycotoxin contamination; Abbas *et al*. (2005) reported that some years favored aflatoxin production while other years appeared to favor FBs production. Logistic regression modelling of cropping systems to predict FBs contamination in maize, based on 438 maize samples collected in five regions of Northern Italy in a six years period (2002-2007), explained around 69% of variability with major roles for longitude, maturity class, and growing weeks (Battilani *et al*., 2008).
Activities performed by the Research Groups involved in the present Project, were coordinated by CREA-CI Bergamo (WP0) and organized in three Work Packages (WPs) as described in Figure 1.

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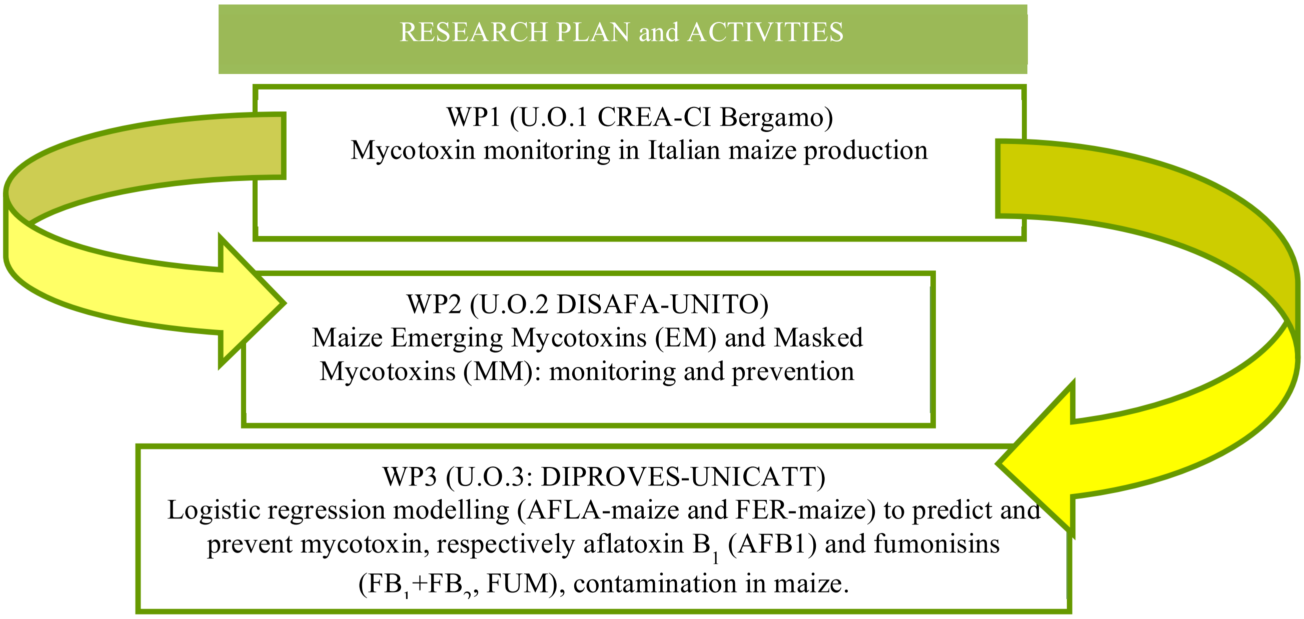

Fig. 1: RQC-MAIS synthetic research plan and Partner interaction in the frame of some selected activities.
Fig. 1: Sintesi del programma di ricerca del progetto RQC–MAIS e interazione tra i Partners nell'ambito di alcune attività selezionate.

Materials and Methods

WP1: MYCOTOXIN MONITORING IN ITALIAN MAIZE PRODUCTION.

Within the project RQC-Mais the CREA monitors large-scale enterprise of samples included in the "agronomic trials - varietal network". In addition, were monitored commercial consignments from numerous storage centers to assess the contamination of the main mycotoxins in the early stages of storage and preservation. The current study was undertaken in Northern Italy, the core area of maize production (Piemonte, Lombardia, Veneto, Friuli Venezia Giulia, Emilia Romagna).

Ridascreen ELISA test kits were used for analyses. Mycotoxin extraction and tests were performed according to the manufacturer's instructions.

WP2: MAIZE EMERGING MICOTOXINS (EM), MONITORING AND PREVENTION.

A monitoring was carried out on 200 commercial maize lots from 4 Regions (Piemonte, Lombardia, Veneto, Emilia Romagna) collected during the period 2012-2015. In addition, a series of field experiments have been conducted in North West Italy in order to evaluate the effect of different crop practices on the contamination of emerging mycotoxins in common and durum wheat and in maize grains. All the experiments have been carried out under naturally-infected field conditions and the following agricultural practices have been considered: hybrids with different susceptibility, planting time, soil tillage, planting time and density, N fertilization and insect control trough insecticide. Detection and quantification of mycotoxins was performed through a multi-mycotoxin method (Malachova et al., 2014).

WP3: DEVELOPMENT OF PREDICTIVE MODELS.

The activity of WP3 can be shared in 3 topics: 1. Validation of predictive models FER-maize and AFLA-maize.
2. Improvement of FER-maize e AFLA-maize 3. Definition of not genetic markers for resistance to toxigenic fungi

- *Validation of predictive models FER-maize and AFLA-maize*

Daily meteorological data of temperature (T, °C), relative humidity (RH, %) and rain (R, mm) has been used as main input for predictive models developed by DIPROVES-UNICATT. Using meteorological data as input, these predictive models give as output a probability of contamination with AFB_1 and FB_1+FB_2 in maize grain at harvest above the legal limit fixed, respectively, at 5 µg and 4000 µg for 1Kg. Using data collected in WP1, model predictions were compared to contamination detected. Validation of these models confirmed good results and the good opportunity to use prediction as support for farmers and other stakeholders.

- *Improvement of predictive models FER-maize and AFLA-maize*

Data on mycotoxin contamination in different maize hybrids were collected in WP1. Differences were observed, also due to the cropping system adopted. This information is under elaboration in order to improve model predictions. Combining this input to meteorological data it is expected an improvement in prediction performances.

- *Definition of not genetic markers for resistance to toxigenic fungi*

Different contamination levels were detected in hybrids sampled in WP1. The two hybrids showing the maximum difference were selected and analysed with a metabolomics approach in order to define not genetic markers for resistance. Data are still under elaboration. It is expected they can confirm and improve results obtained in previous studies and make available new

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non genetic markers for resistance to toxigenic fungi that can be applied to all maize hybrids entering the market. This could be very useful also to include the "hybrid" in predictive models.

Results and Discussion

WP1: Mycotoxin monitoring in Italian maize production.
STORAGE CENTERS MONITORING NETWORK.
A total of 1076 representative grain samples were collected, after dry processing, over a 3-year period (2014–2016) from about 50 storage centers. These storage centers represent about 8–9% of Italian maize production and are distributed across the principal maize cultivation areas. From each storage centre, 8–10 samples were collected each year. A dynamic grain sampling strategy was performed on the product in motion to obtain a representative sample.
Fusarium verticillioides is endemically present in Italy, because of specific adaptation to the environmental and climatic conditions (Locatelli et a. 2016a). Particular climatic anomalies favour the presence of *Aspergillus flavus* (Locatelli et al., 2016b) or *Fusarium graminearum* (Locatelli et al., 2015 and Locatelli et al., 2017). Monitoring activities conducted through a network of sampling stable over the years, is an essential tool for the management of domestic stocks and to highlight new mycotoxin alerts.
AGRONOMIC TRIALS – VARIETAL NETWORK
The monitoring program supported by the Agronomic trials – varietal network in which 10 fields, selected in the principal cultivation areas in Northern Italy (Piemonte, Lombardia, Veneto, Friuli Venezia Giulia, Emilia Romagna) where chosen. During 2015-2016 in each location seven hybrids, representative of the main FAO classes (500 – 600 – 700) were grown. The experimental design was a split splot design with four replicates The results obtained monitoring mycotoxin accumulation in agronomic trials - varietal network indicate that this may be an useful strategy for the prevention and containment of mycotoxin development.

WP2. (U.O.2 DISAFA-UNITO): Maize Emerging Micotoxins (EM): monitoring and prevention
Applying the multi-toxin method 25 of the most abundant mycotoxins were detected in maize samples: fumonisin B1, B2, B3, B4 (FBs), fusaric acid (FA), bikaverin (BIK), beauvericin (BEA), moniliformin (MON), fusaproliferin (FUS), equisetin (EQU), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-G), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), zearalenone (ZEA), zearalenone-4-Sulfate (ZEA-4S), culmorin (CULM), butenolide (BUT), aurofusarin (AUR), and aflatoxin B1, B2, G1, G2 (AFs), ochratoxin A (OTA) and B.

Tab. 1: Mycotoxin contamination in maize commercial samples collected in the 4 Regions of North Italy monitored during the period 2012-2015.
Tab. 1: Contaminazione di micotossine in campioni commerciali di mais raccolti nelle 4 regioni del Nord Italia monitorate nel period 2012-2015.

Main fungi producers	Mycotoxin	2012	2013	2014	2015	Positive samples[1]
		μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	%
Fusarium section Liseola	FBs	8997	6151	15040	9456	100
	FA	356	1236	492	230	100
	BIK	294	853	175	102	100
	BEA	187	195	135	101	100
	MON	852	344	505	574	100
	FUS	959	1551	1346	875	94
Fusarium section Gibbosum	EQU	40	55	59	15	90
Fusarium section Discolor and Roseum	DON	419	2923	3007	257	77
	DON-3-G	138	595	1247	89	89
	CULM	197	2621	970	94	79
	ZEA	26	367	490	24	85
	BUT	60	410	592	117	85
	AUR	194	4099	9642	239	85
Aspergillus	AFs	40	14	1	5	53
Aspergillus, Penicillium	OTA	2	nd	nd	Nd	2

[1] Percentage of sample above the limit of quantification considering 94 maize samples collected in 2 growing seasons. nd. not detected.

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The relative percentage of presence of mycotoxins produced by Fusarium section Liseola (FBs, FA, BIK, BEA, MON, FUS) in maize commercial lot samples was 100% (Table 1). The occurrence of other mycotoxins was clearly influenced by growing season, with remarkable and hazardous AFs contamination values in 2012. The content of mycotoxins produced by Fusarium spp. of Liseola section, such as FBs, MON, FUS, FA, BIK and BEA was significantly reduced by insecticide application to reduced insect ear injuries, while it was increased by N stress and late planting times. Conversely, DON, DON-3-G, ZEA, CULM, AUR and BUT contents, produced by Fusarium spp. of Discolor and Roseum sections, were not affected significantly by the presence of insect injuries, while were clearly related to excess of N fertilization, high plant density and no tillage conditions.

WP3. (U.O.: DIPROVES-UNICATT): Development of predictive models

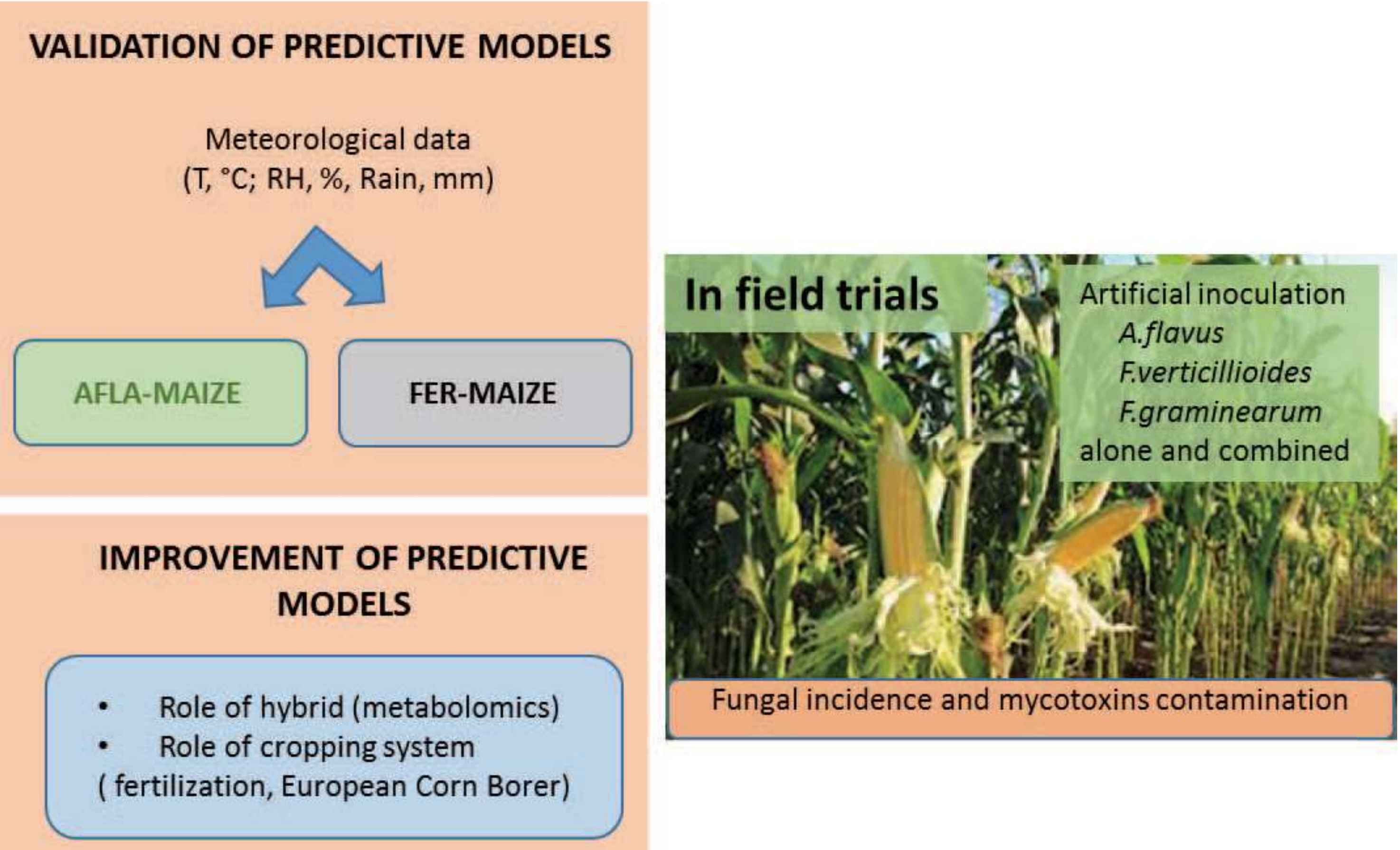

Fig. 2: WP 3 Description of activities developed for validation and improvement of predictive models
Fig. 2: WP3 Descrizione delle attività sviluppate per la validazione e il miglioramento dei modelli predittivi

Validation of predictive models (*Fig. 2*), run on a limited data set, gave good results; contamination above the legal limit was commonly correctly predicted. Hybrids showed a significant role on mycotoxin contamination, at least for fumonisins. Relevant variability in hybrid behaviour between growing areas was furthermore observed. Anyway, hybrid effect, if confirmed with the second-year data, will be included in predictive models to improve their performances.

Conclusions

Available data for the incidence of mycotoxins, in particular for EM, on maize production in Italy are limited and irregular; additionally, no national database for collecting information to predict annual risk exposure is active. Therefore, a systematic effort to monitor the levels of contaminants in maize grain production is needed.

RQC-Maize project highlighted to possibility to combine research expertise useful to develop a national plan devoted to maize crop chain safety improvement in order to increase feed and food industry competitiveness.

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