Bari headquarters

Institute of Sciences of Food Production National Research Council of Italy
(ISPA-CNR)
Via G. Amendola 122/O - 70126 Bari, Italy

(***www.ispa.cnr.it***)

Introduction and general description of ISPA activities

National Research Council of Italy (CNR)

- **CNR is a public organization**; its duty is **to carry out, promote, spread, transfer and improve research activities in the main sectors of knowledge** and of its applications for the scientific, technological, economic and social development of the Country.
- To this end, the activities of the organization are divided into macro areas of interdisciplinary scientific and technological research, concerning several sectors: **biotechnology, medicine, materials, environment and land, information and communications, advanced systems of production, judicial and socio-economic sciences, classical studies and arts**.
- **CNR is distributed all over Italy through a network of institutes** aiming at promoting a wide diffusion of its competences throughout the national territory and at facilitating contacts and cooperation with local firms and organizations.

DEPARTMENTS

- Earth system science and environmental technology
- **Agri-Food Biosciences**
- Biomedical sciences
- Chemistry and Material Technologies
- Physical sciences and technologies of matter
- Engineering, ICT and technologies for energy and transports
- Humanities and social sciences, cultural heritage

*CNR is composed by **7 Departments**, with programming, coordination and control tasks and by **109 Institutes**, where research activities are performed.*

Institute of Sciences of Food Production

ISPA distribution in the Italian territory

Turin

Staff	**12**
Researchers	7
Technicians	4
Administrative	1

Milan

Staff	**3**
Researchers	3

Bari headquarters

Staff	**57**
Researchers	37
Technicians	16
Administrative	4

Sassari

Staff	**25**
Researchers	9
Technicians	13
Administrative	3

Lecce

Staff	**18**
Researchers	13
Technicians	3
Administrative	2

ISPA-CNR

Territorial Units	**5**
Staff	**115**
Researchers	**69**

Director: Dr. Antonio F. Logrieco

Microbiology and quality of food production

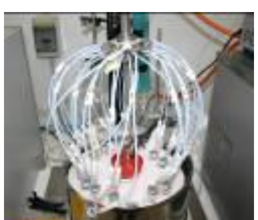
- Dairy products
- Meat products
- Fermented beverages
- Bakery products and Pasta
- Fermented vegetable products
- Probiotic products

Biotechnologies for food quality

- Metabolic pathways engineering to improve quality and nutritional value of plant products
- Biotechnological production of proteins and plant secondary metabolites for agri-food industry
- Functional foods
- Wine technology

Food safety and innovative methods for food analysis

- Evaluation of food quality, typicalness, traceability and safety
- Analysis of food contaminants (mycotoxins, toxigenic fungi, allergens)
- Control strategies for reduction of mycotoxins and toxigenic fungi
- Chromatographic, spectroscopic and molecular methods; biosensors
- Analysis of biomarkers of exposure to mycotoxins



Sustainable production systems and quality of horticultural crops

- Microorganisms and their metabolites for biological control
- Preservation and exploitation of typical horticultural products
- Biochemical composition of horticultural products
- Agronomical techniques and
- Soil-less cultivation of horticultural crops





Processing, preservation and packaging technologies for horticultural crops

- Control of postharvest losses and quality maintenance of horticultural products
- Postharvest technology of horticultural crops
- Postharvest physiology and pathology of fruits and vegetables

Heritage

Landscapes

Good food

ISPA

National Research Council

INSTITUTE OF SCIENCES
OF FOOD PRODUCTION

Welcome!

- **A) Food safety: Mycotoxin issue as key study**

- **B)Innovative and integrated managment of mycotoxins along chain**

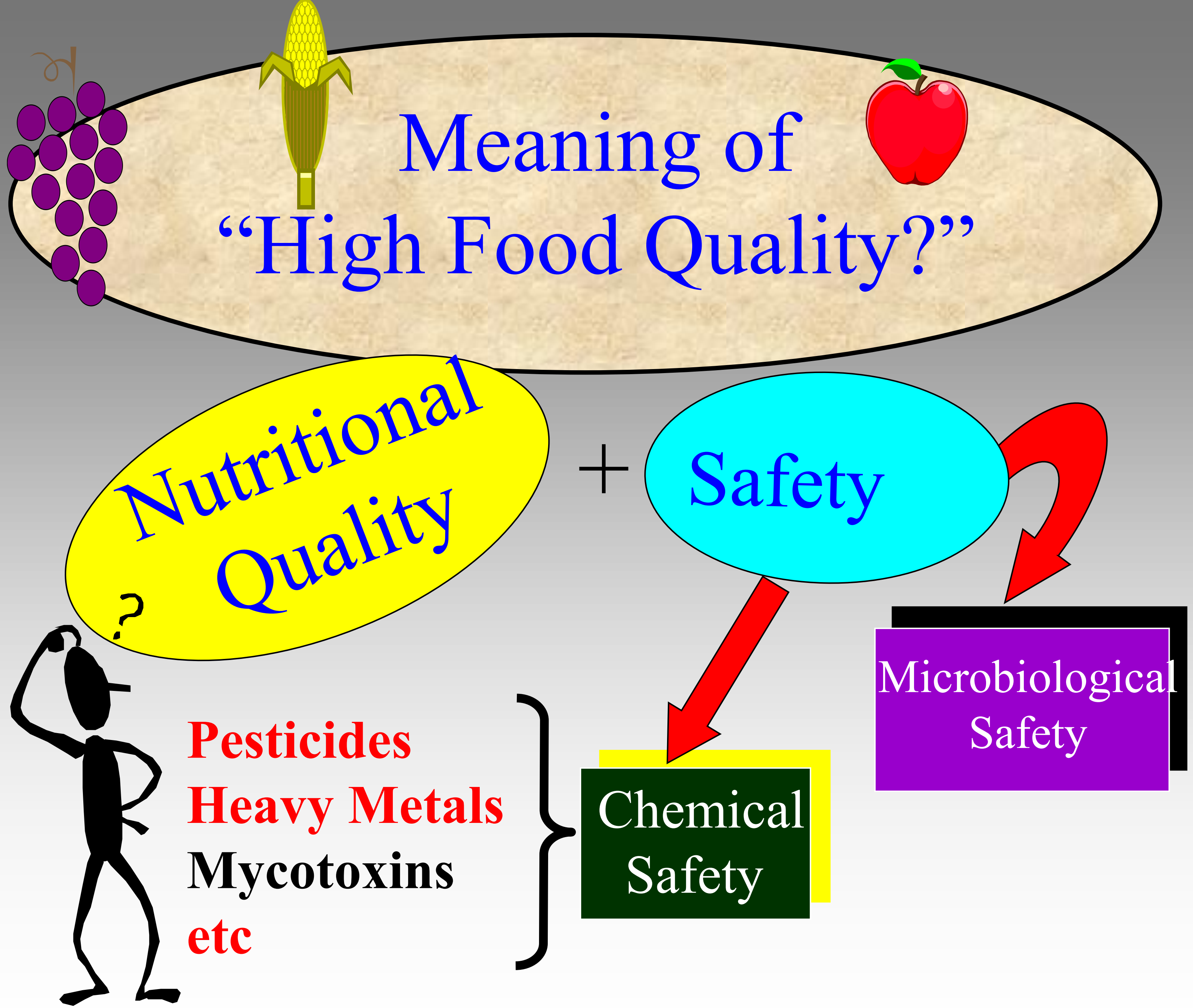
- **C) MycoKey project: EU Horizon 2020**

Mycotoxin contamination: a global concern

Antonio F. Logrieco - CNR ISPA

ISPA
ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

MycoRed

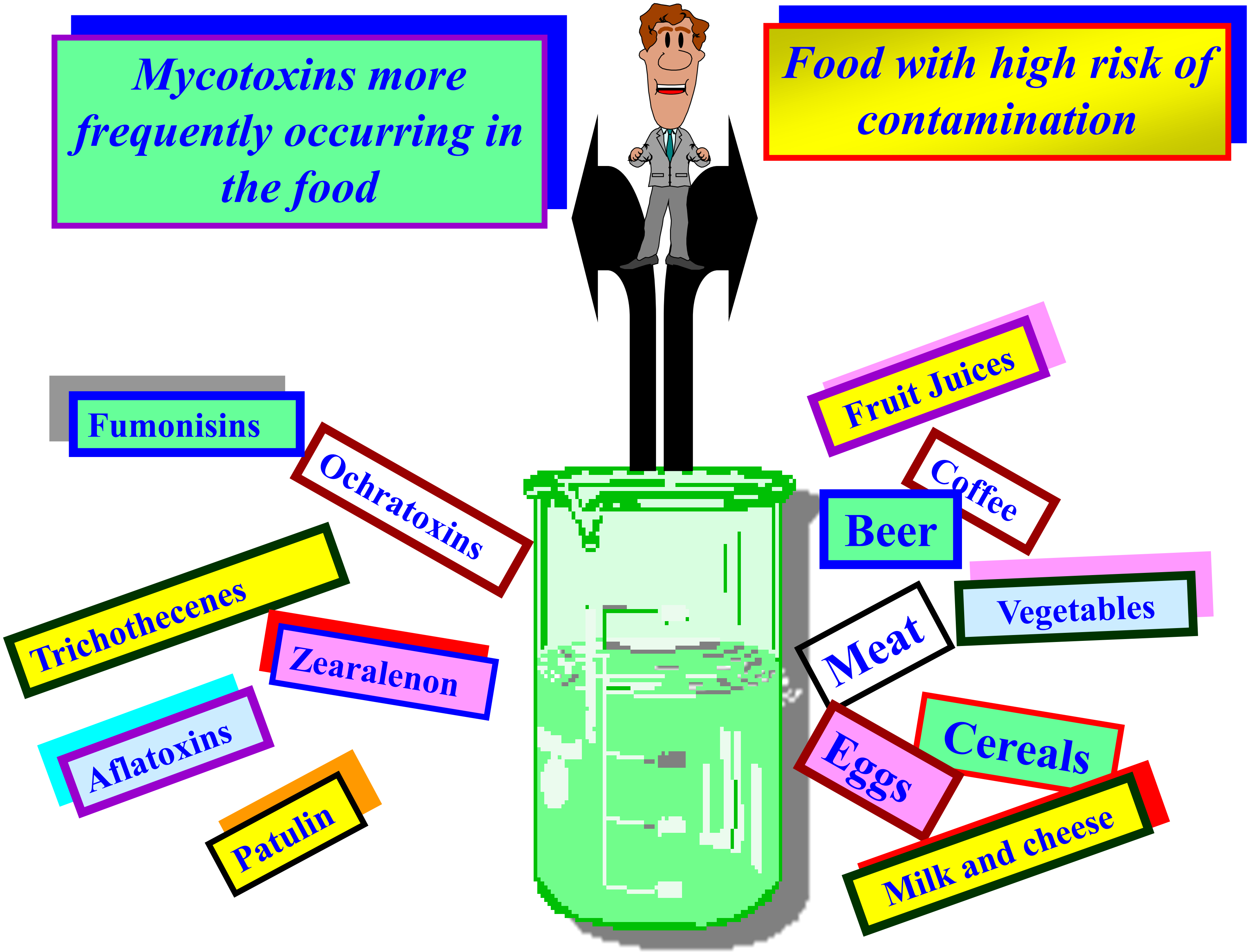

Meaning of
"High Food Quality?"
Nutritional
Quality
+
Safety
Microbiological
Safety
Pesticides
Heavy Metals
Mycotoxins
etc
Chemical
Safety

WHAT ARE MYCOTOXINS?

- FAO data: In the world, more than 25 % of foods are considered significantly contaminated by mycotoxins
- Mycotoxins are secondary metabolites produced by molds
- **They:**
  - **characterize the genera, the species or the producer strain**
  - **are bio-synthetized by few reactions**
  - **are classified in specific groups depending on their biosynthetic pathway**
  - **are compounds chemically heterogeneous**
  - **have most biological activities , both in animals and in men**

WHAT ARE MYCOTOXINS?

- More than 400 different types of mycotoxins are known about 10% of which occur in foods, being the main source for human exposure;
- Resistance to common decontamination treatments;
- Several genera could produce the same mycotoxin;
- The same genus could produce several mycotoxins
- Cereals and derivate products, vegetables, dried fruit, milk, cheeses, meet and eggs may introduce mycotoxins on the table



by Alberto Ritieni 2000

Primary Acute Micotoxicoses

➢ **vascolar system**	**vascolar fragility, hemorhage**
➢ **respiratory system**	**pulmonary edema**
➢ **digestive system**	**diarrhoea, hepatotoxicity, hepatic necrosis**
➢ **nervous system**	**tremors, leukoencephalomalacia**
➢ **skin**	**photosensitivity, necrosis, desquamation**
➢ **urinary system**	**nephrosis, uremia**
➢ **reproductive system**	**infertility, oestrum disorders**

Primary Chronic Micotoxicoses

Pathologies not macroscopic which symptoms are:

- *Decreased milk production*
- *Decreased productivity*
- *Decreased egg production*
- *Lower quality of products*
- *Decreased growth*

CLASSIFICATION OF SOME MYCOTOXINS ACCORDING WITH CARCINOGENIC RISK FOR HUMANS (IARC, 1993)

Gruppo 1	**Carcinogenic for humans**	**Aflatoxins B_1, B_2, G_1, G_2**
Gruppo 2B	**Carcinogenic for animal and potential carcinogenic for humans**	**Aflatoxin M_1** **Ochratoxin A** **Fumonisins**

from: *International Agency for Research on Cancer*. *Monographs on the Evaluation of Carcinogenic risk to humans*. IARC Lyon, France, VOL. 56, 1993, 523-524

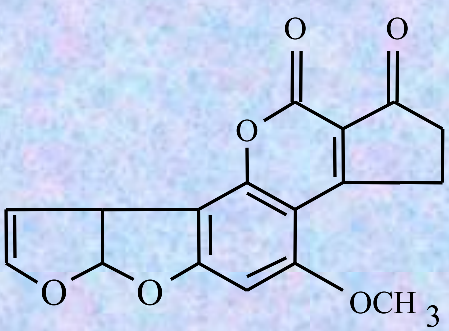
MAIN MYCOTOXINS OCCURRING ON FOOD AND RELATED FUNGAL SPECIES PRODUCERS

MYCOTOXINS	FOOD	FUNGAL SPECIES
Aflatoxins B_1, B_2, G_1, G_2	maize, peanuts, spices, dried fruit	*Aspergillus flavus, A. parasiticus*
Aflatoxin M_1	milk, eggs, cheese	*Aspergillus flavus, A. parasiticus*
Ochratoxin A	cereals, coffee, wine, beer,	*Aspergillus ochraceus, A. carbonarius, A. niger, Penicillium verrucosum*
Patulin	apple juice	*Pecillium expansum*
Deoxynivalenol	cereals	*Fusarium graminearum, F. culmorum*
Zearalenon	maize, maize by-products	*Fusarium graminearum, F. culmorum*
Fumonisins	maize, maize by-products	*Fusarium verticillioides (F. moniliforme), F. proliferatum*

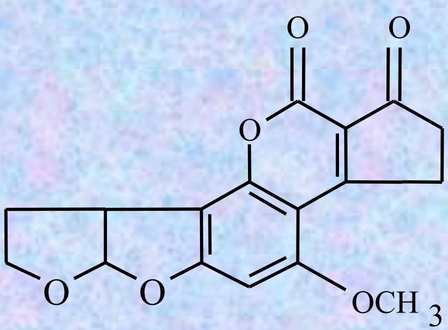
Translation of oldest food law

”Thou shalt not taint the fat or the bread of thy neighbour, neither shalt thou bewitch the fat or the bread of thy neighbour”

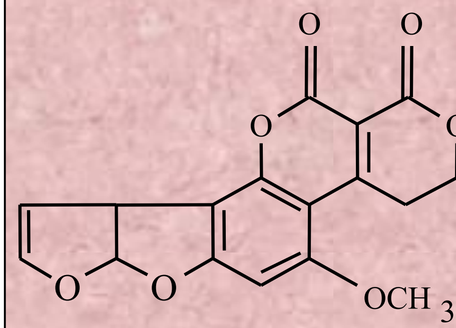
AFLATOXINS



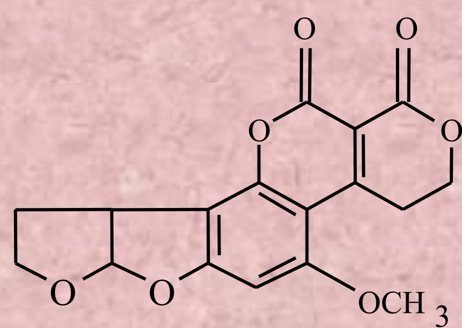
Aflatoxin B1



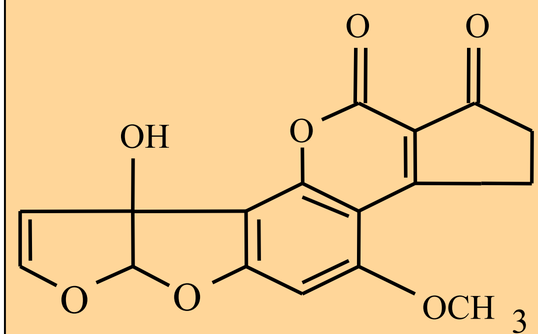
Aflatoxin B2



Aflatoxin G1



Aflatoxin G2

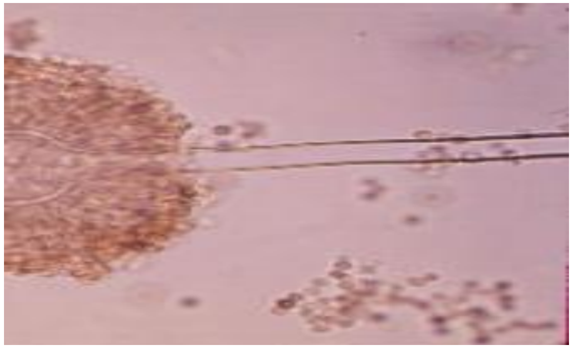


Aflatoxin M1

Aflatoxins were identified and isolated in the ’60s in England. They occur in nuts, peanuts butter and oleaginous seeds. They can contaminate also cereals, milk, and cheese.

AFB_1, is at the moment the only mycotoxin of which was clearly reported the correlation with hepatocarcinome in some areas whit low both social and economical levels.

Aspergillus spp.
Aflatoxin producers

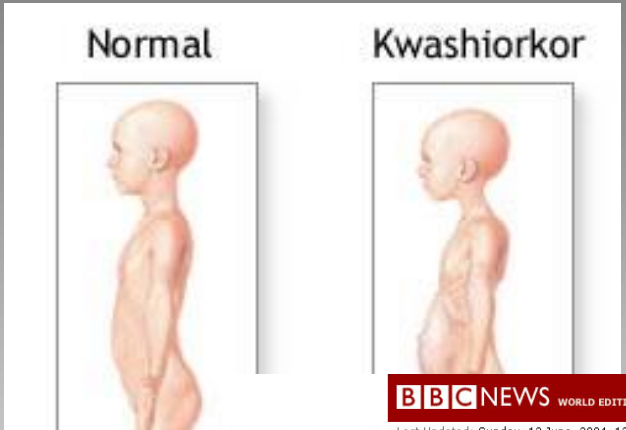


Ingestion effects of aflatoxins B1 on a chick

by Alberto Ritieni 2000

Human health impact of aflatoxin

- **Acute toxicity in humans rare but**
  - 40 deaths in 1974 (India)
  - 13 deaths in 1990 (Malaysia)
  - 14 deaths in 2001 (Kenya)
  - 125 deaths in 2004 (Kenya)
  - 39 deaths in 2010 (Nigeria)
  - ………
- **Sub-acute and chronic effects in humans**
  - liver cancer, chronic hepatitis, jaundice, enlarged liver (hepatomegaly) and cirrhosis
  - suppressed immune system
  - increased mortality with Kwashiorkor



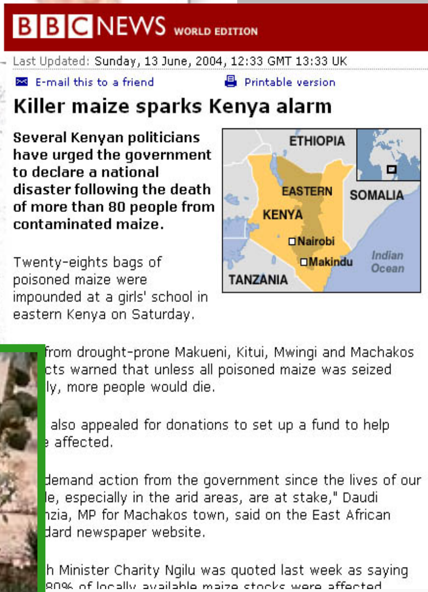
RELIEF

Spoilt maize threatens schools food programme

By LUCAS BARASA

A school-feeding programme for 40,000 pupils in some of Nairobi's slums has been thrown into disarray by contaminated relief food.

Hundreds of bags of yellow maize and peas donated by the US government



BBC NEWS WORLD EDITION

Last Updated: Sunday, 13 June, 2004, 12:33 GMT 13:33 UK

E-mail this to a friend Printable version

Killer maize sparks Kenya alarm

Several Kenyan politicians have urged the government to declare a national disaster following the death of more than 80 people from contaminated maize.

Twenty-eights bags of poisoned maize were impounded at a girls' school in eastern Kenya on Saturday.

…from drought-prone Makueni, Kitui, Mwingi and Machakos …cts warned that unless all poisoned maize was seized …ly, more people would die.

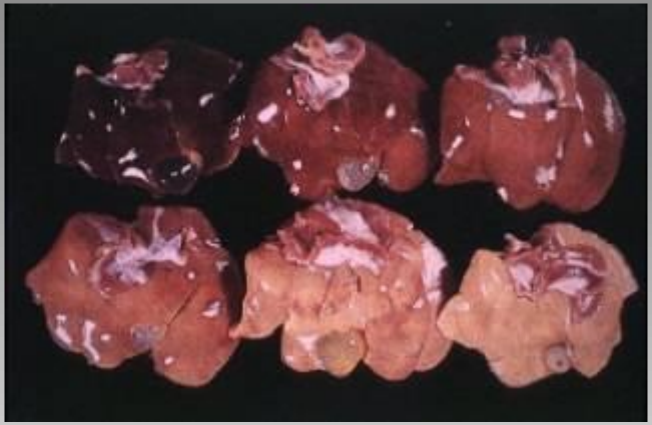
… also appealed for donations to set up a fund to help … affected.

…demand action from the government since the lives of our …le, especially in the arid areas, are at stake," Daudi …nzia, MP for Machakos town, said on the East African …dard newspaper website.

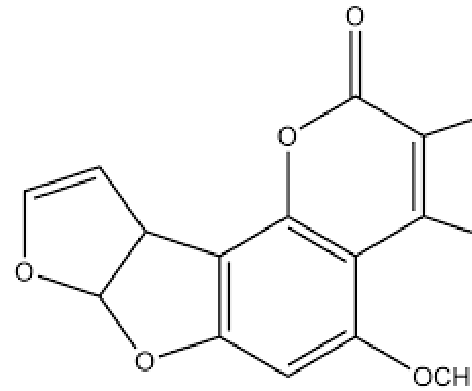
…h Minister Charity Ngilu was quoted last week as saying …

Animal Health Impact of Aflatoxin

- **Livestock and poultry losses**
  - liver damage including cancer
  - recurrent infection due to immune system suppression
  - reduced growth rate
  - losses in feed efficiency
  - decreased milk and egg yield
  - embryo toxicity (reduced reproductivity)
  - death (cattle, turkey, poultry, swine..)



Aflatoxin B_1



- Major mycotoxin contaminant (RASFF, 2015) in nuts, cereal grains
- Carry over in milk as AFM_1

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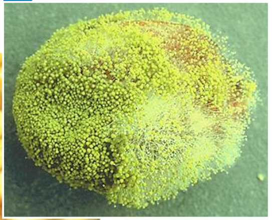
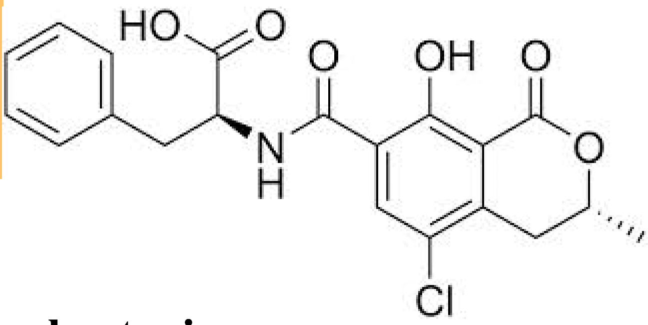
Trade losses due to aflatoxins

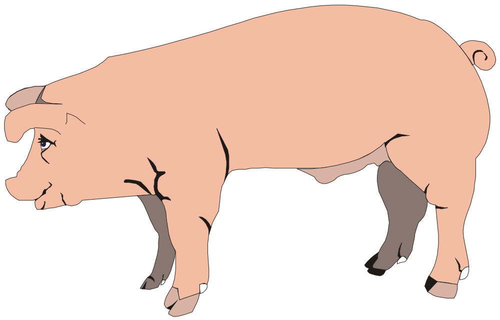



- **Export compliance with food safety and quality standards.**
- **CODEX standard: 20 ppb; EU: 2 ppb**
- **African exports to EU (51%) & the US (22%)**
- **Estimated US$670 million in lost trade for 9 African countries due to EU reduced regulatory levels for aflatoxins**
- **Trade reduction: 46%.**
- **Best quality exported; poorer quality consumed domestically.**

Ochratoxin A: Aspergillus/Penicilium
Toxic effects



ANIMALS



Activities: Carcinogenic, nephrotoxic, gene-toxic, mutagenic, teratogenic, fetus-toxic, immunosuppressive.

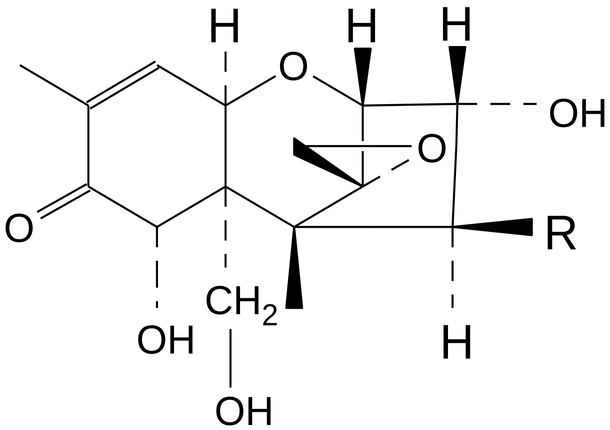
HUMANS

OTA is frequently found in human blood. It has been associated to Nephropathy Endemic of Balkans, and it is suspected to cause tumors of urinary system and interstizial chronic nephritis in Northern- Africa

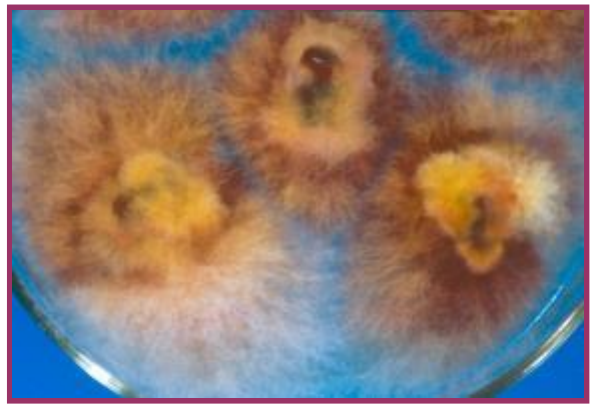
Type B Trichothicenes

F. graminearum + F. culmorum

Deoxynivalenol / Nivalenol

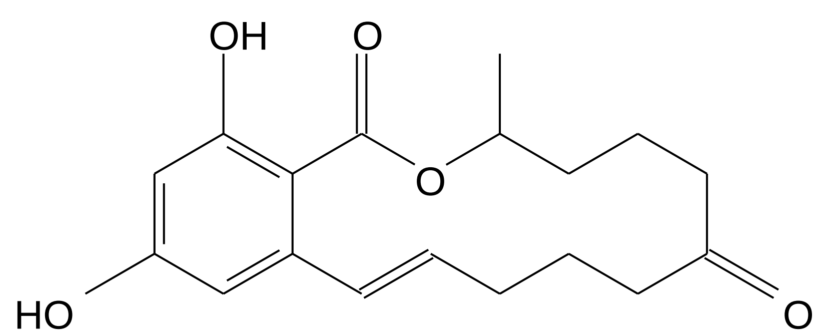


Feed Refusal and Emetic Syndromes

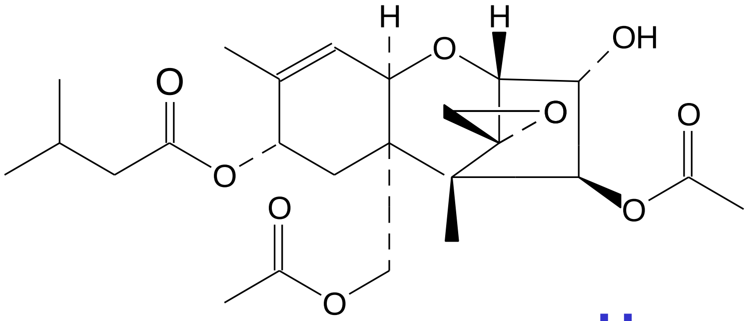


Estrogenic Syndrome

Zearalenone



Type A Trichothicenes



F. sporotrichioides + F. poae+ F. Langsethiae

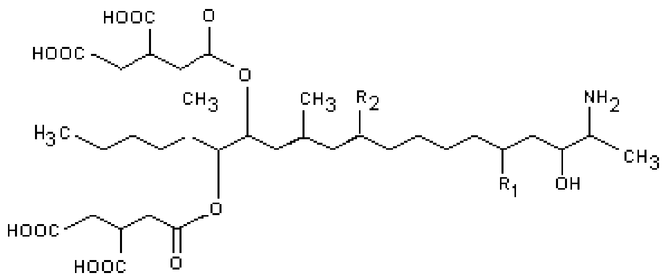
T2-toxins and Diacetoxyscirpenol

Hemorrhagic Syndromes

Alimentary Toxic Aleukia in Humans

Moldy Corn Toxicosis in Animals

FUMONISINS

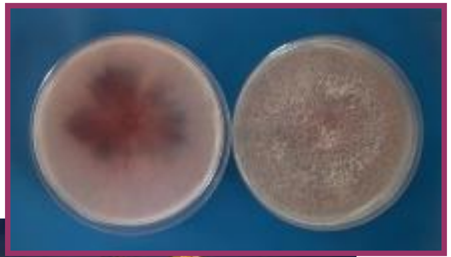


	R_1	R_2
Fumonisin B_1	OH	OH
Fumonisin B_2	OH	H
Fumonisin B_3	H	OH
Fumonisin B_4	H	H

Historical background: **Butler, in 1902, described a horse's disease called equine Leucoencephalomalacia (ELEM)**

F. verticillioides **that produce FBs has been associated with other diseases such as:**

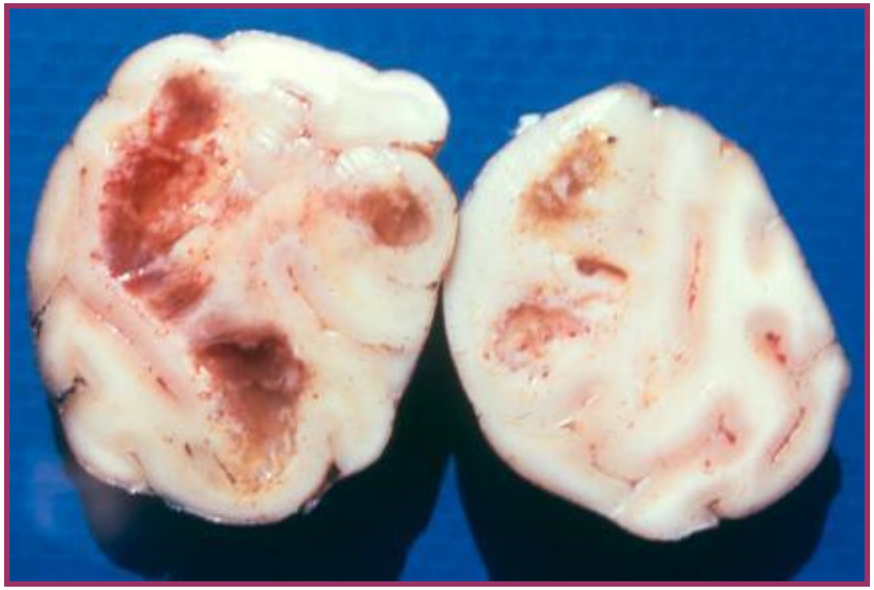
-pulmonary edema in pigs
-oesophageal cancer in humans
-nephrosis and hepatosis in sheep
-cardiac thrombosis hepatic cirrhosis in rats

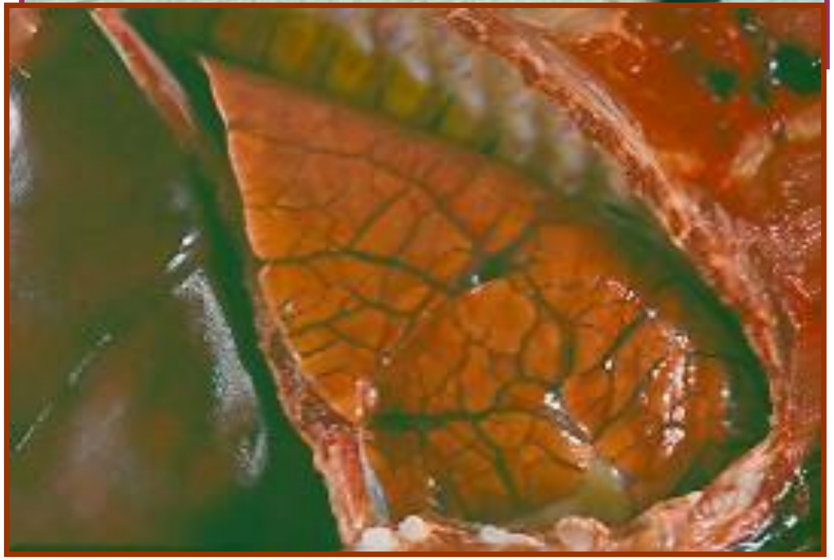


Mechanisms of action:

- Disruption of sphingolipid metabolism
- Alterated fatty acid metabolism
- Synergy and interactions

FUMONISIN associated deseases



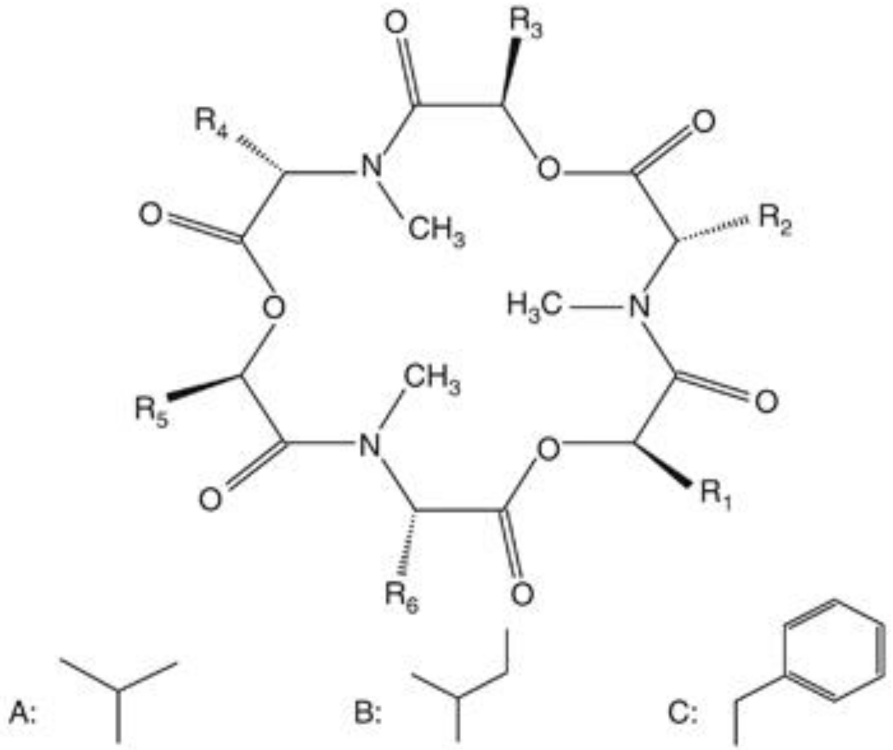


Fumonisin Producing *Fusarium* Species

Fusarium sp.	Fumonisin analogs
<u>Section Liseola</u>	
F. verticillioides	FA_{1-3}, FB_{1-5}, iso-FB_1, FAK_1, FBK_1, $FC_{1,4}$, FP_{1-3}, PH_{1a-b}
F. sacchari	FB_1
F. fujikuroi	FB_1
F. proliferatum	FA_{1-3}, FB_{1-5}, FAK_1, FBK_1, FC_1, FP_{1-3}, PH_{1a-b}
F. subglutinans	FB_1
F. thapsinum	FB_{1-3}
F. anthophilum	FB_{1-2}
F. globosum	FB_{1-3}

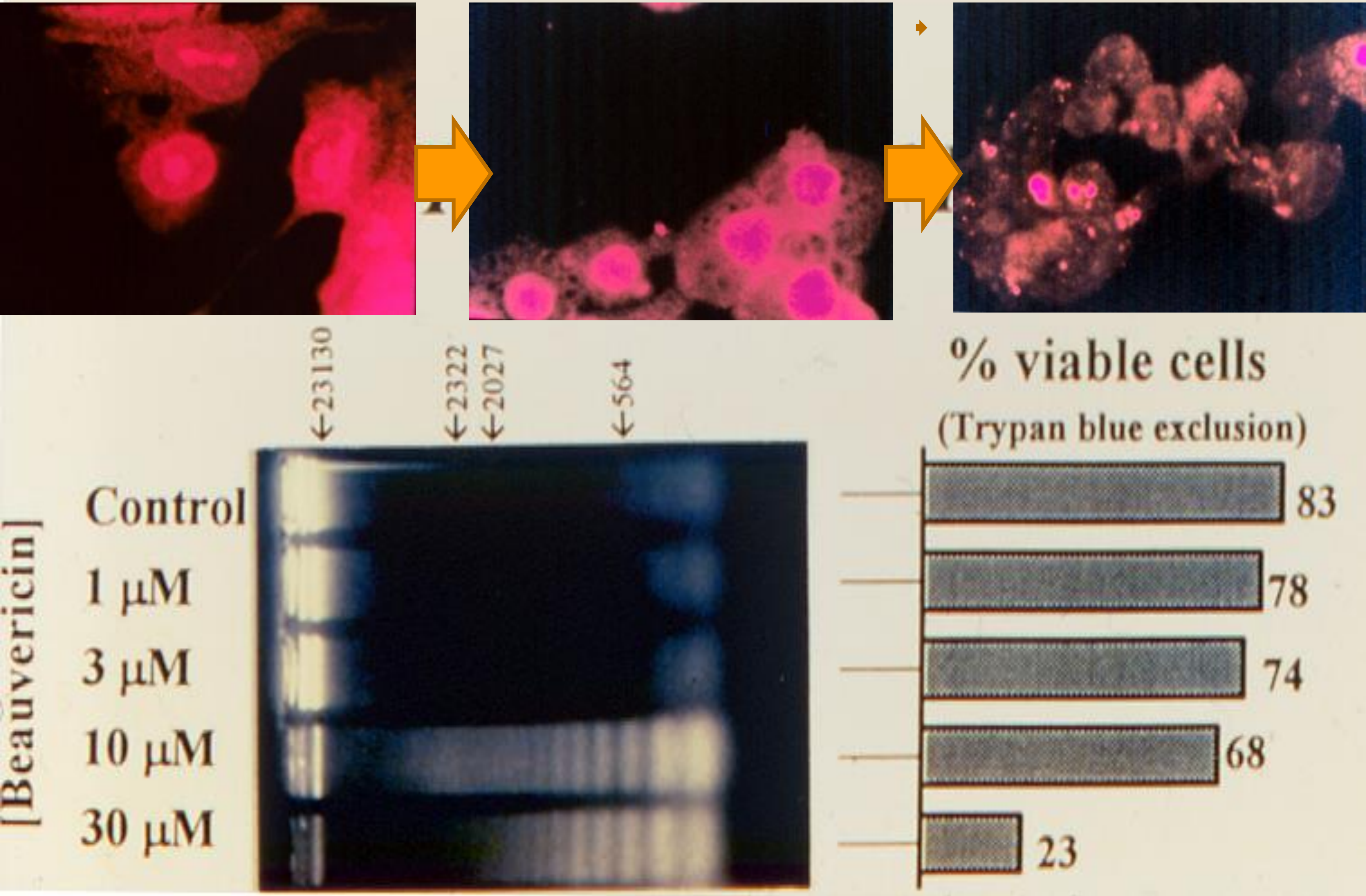
Rheeder et al. 2002. Appl Environ Microbiol 68: 2101-2105

Beauvericin



Compound	R_1	R_2	R_3	R_4	R_5	R_6	$[M+H]^+$
Beauvericin	A	C	A	C	A	C	784
Enniatin H	B	A	A	A	A	A	654
Enniatin I	B	A	A	A	B	A	668
Enniatin MK1688	B	A	B	A	B	A	682

RBL-1 cells



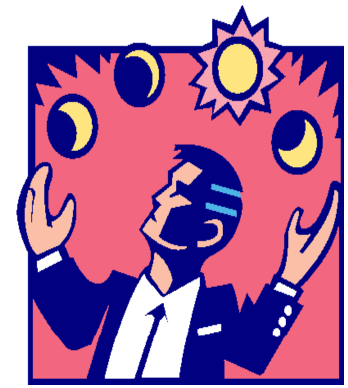
23130
2322
2027
564
[Beauvericin]
Control
1 μM
3 μM
10 μM
30 μM
% viable cells
(Trypan blue exclusion)
83
78
74
68
23

Some Beauvericin biological activities :

Insectidial properties against mosquito larvae

20 µg/mL show 86% of mortality at 48 h

SF9 pupal ovarian cell line of *Spodoptera frugiperda* 10 µM



Potato beetle activity

Antibiotic activity against:

Staphilococcus aureus; Sarcina lutea

Candida albicans; Saccharomyces cerevisia

Brine shrimp larvae

Artemia salina

Toxicity towards human and animal cell lines:	**CC_{50}**	**24h**	
HeLa human cervical carcinoma cells		13	µM
HEP G2 human hepatoma cells	5	µM	
IARC BL-41 human Burkitt's lymphoma cells		2.5	µM
U937 human hystiocytic lymphoma cells		5	µM
RBLS-1 rat basophilic/mast cells	4	µM	
CV-1 monkey kidney non neoplastic fibroblastic		15	µM

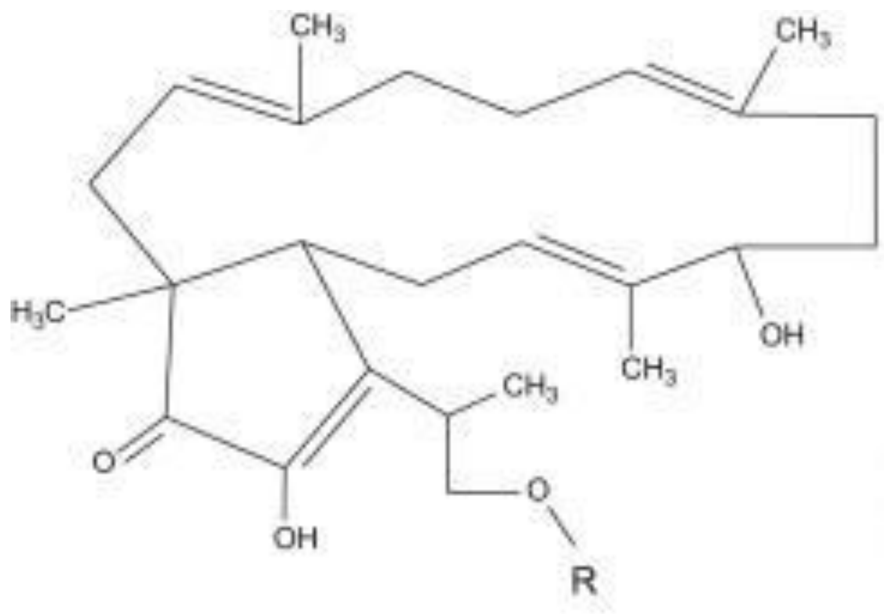
BEA Production and occurrence

Main phytopathogenic *Fusarium* species producing BEA:

- *F. proliferatum* (various hosts)
- *F. fujikuroi* (rice)
- *F. temperatum* (maize & sugar cane)
- F. sambucinum (potato)
- F. avenaceum (wheat)
- F.poae (cereals)
- F. oxysporum (e.g. f.s. melonis; asparagi, dianthi)
- F. solani

Maize infected by *F. proliferatum* and *F. subglutinans* and wheat infected by *F. avenaceum* are the main hosts contaminated by BEA

Fusaproliferin and derivatives



$R_1 = R_2 = R_3 = H$ Deacetilfusaproliferina

$R_1 = R_2 = H$ $R_3 = CH_3CO$ Fusaproliferina

$R_1 = R_2 = R_3 = CH_3CO$ Acetilfusaproliferina

Acephalia

Cephalic dichotomy

Anomalous development of abdomen region

Fusaproliferin

FACTORS OF MYCOTOXIN PENETRATION IN THE FOOD CHAIN

In field

Biological factors
Susceptible plants
Toxigenic Fungi
(Fusarium, Alternaria, Aspergillus)

Environmental factors
Temperature, rainfall
Mechanical damages
Insects Birds Rodent

Harvest time
Harvest methods
Maturation levels

Storage steps

Temperature
Humidity
Previous contaminations
(*Aspergillus & Penicillium*)

Distribution

HUMANS ← ANIMALS

TOXIGENIC FUNGI -MYCOTOXINS

Wheat and Maize Products

Wheat

Fusarium Head Blight

major species

Fusarium graminearum

F. culmorum

F. avenaceum

F. poae

minor species

F. cerealis
(syn F. crookwellense)

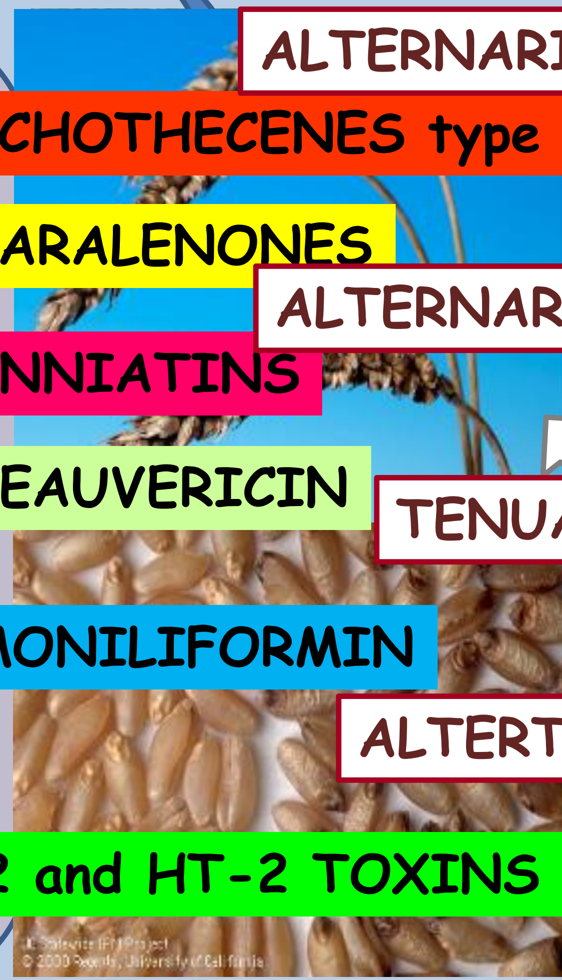
F. sporotrichioides

F. tricinctum

F. acuminatum

F. equiseti

F. langsethiae



TRICHOTHECENES type B

ZEARALENONES

ENNIATINS

BEAUVERICIN

MONILIFORMIN

T-2 and HT-2 TOXINS

Black point

Alternaria spp.

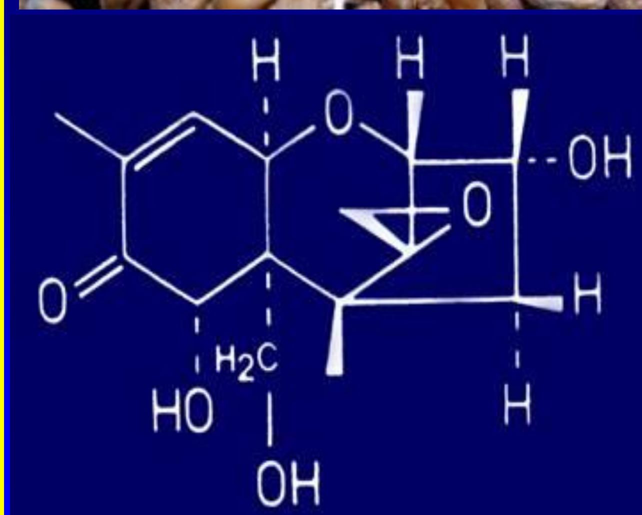
ALTERNARIOL

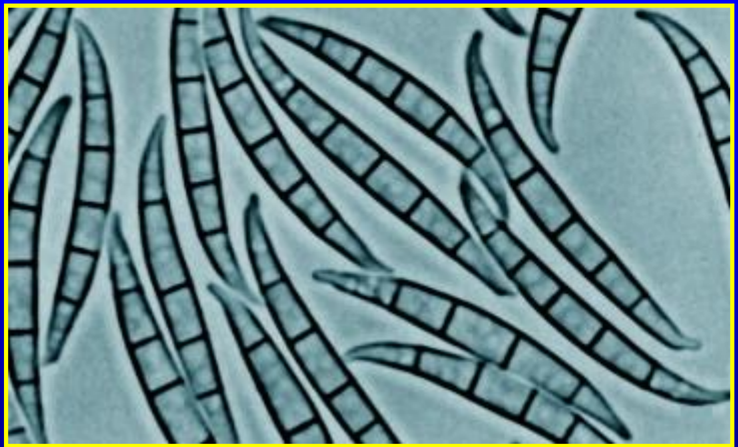
ALTERNARIOL METHYL ETHER

TENUAZONIC ACID

ALTERTOXINS

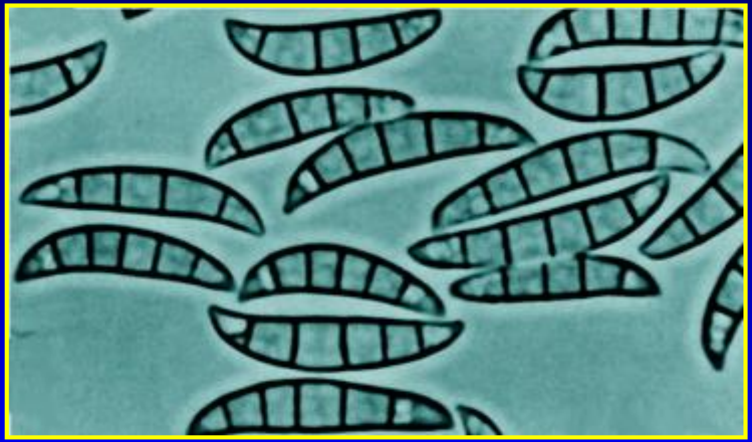
Fusarium head blight (FHB) of wheat and DON/NIV and ZEA





Fusarium graminearum

Fusarium cerealis

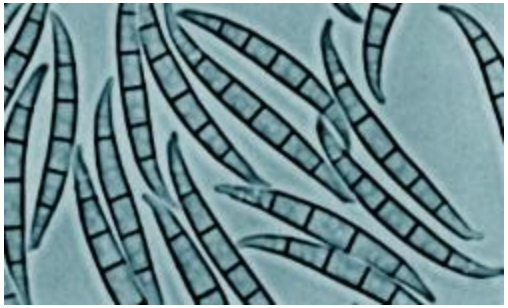


Fusarium culmorum

Fusarium avenaceum

Main toxins produced by *Fusarium graminearum*

- Deoxynivalenol
- Monoacetyl-deoxynivalenols (3- and 15AcDON)
- Nivalenol
- Fusarenone X
- Zearalenone
- Zearalenols (α and β isomers)

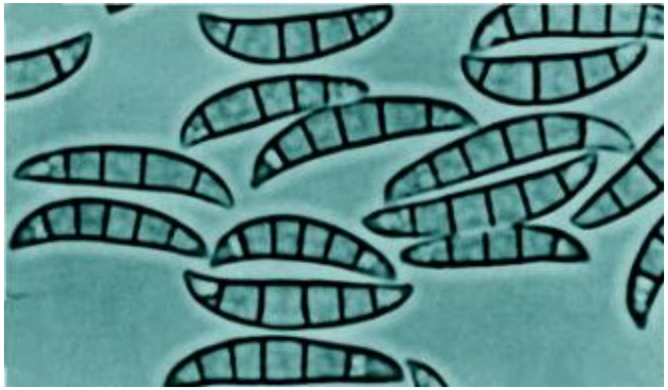


Main toxins produced by *Fusarium avenaceum*

- Moniliformin
- Beauvericin
- Enniatins (B, B1 and A1)
- Fusarin C
- Antibiotic Y

Main toxins produced by *Fusarium culmorum*

- Deoxynivalenol
- Nivalenol
- Fusarenon X
- Zearalenone
- Zearalenols

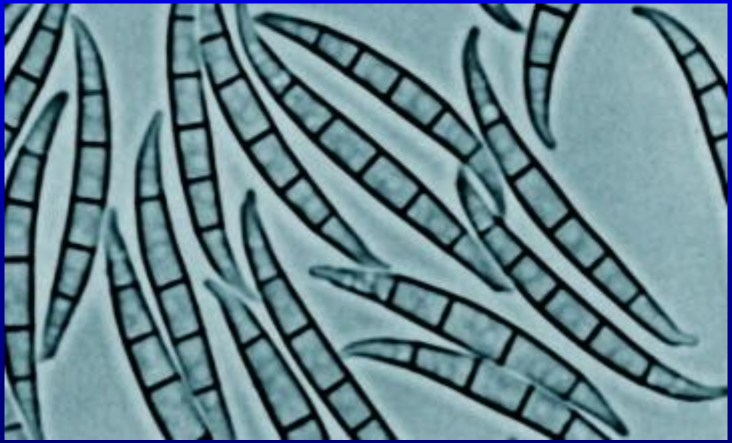


Main toxins produced by *F. poae* and related species

- ***F. poae***: Nivalenol, Fusarenone X, diacetoxyscirpenol

  Beauvericin, Enniatin B
- ***F. langsethiae:*** Neosolaniol, T-2 and HT-2 toxin,
- ***F. sporotrichioides:*** Neosolaniol, T-2 and HT-2 toxin

F. graminearum (anamorph *Gibberella zeae*) causes scab or head blight of wheat and other small grains

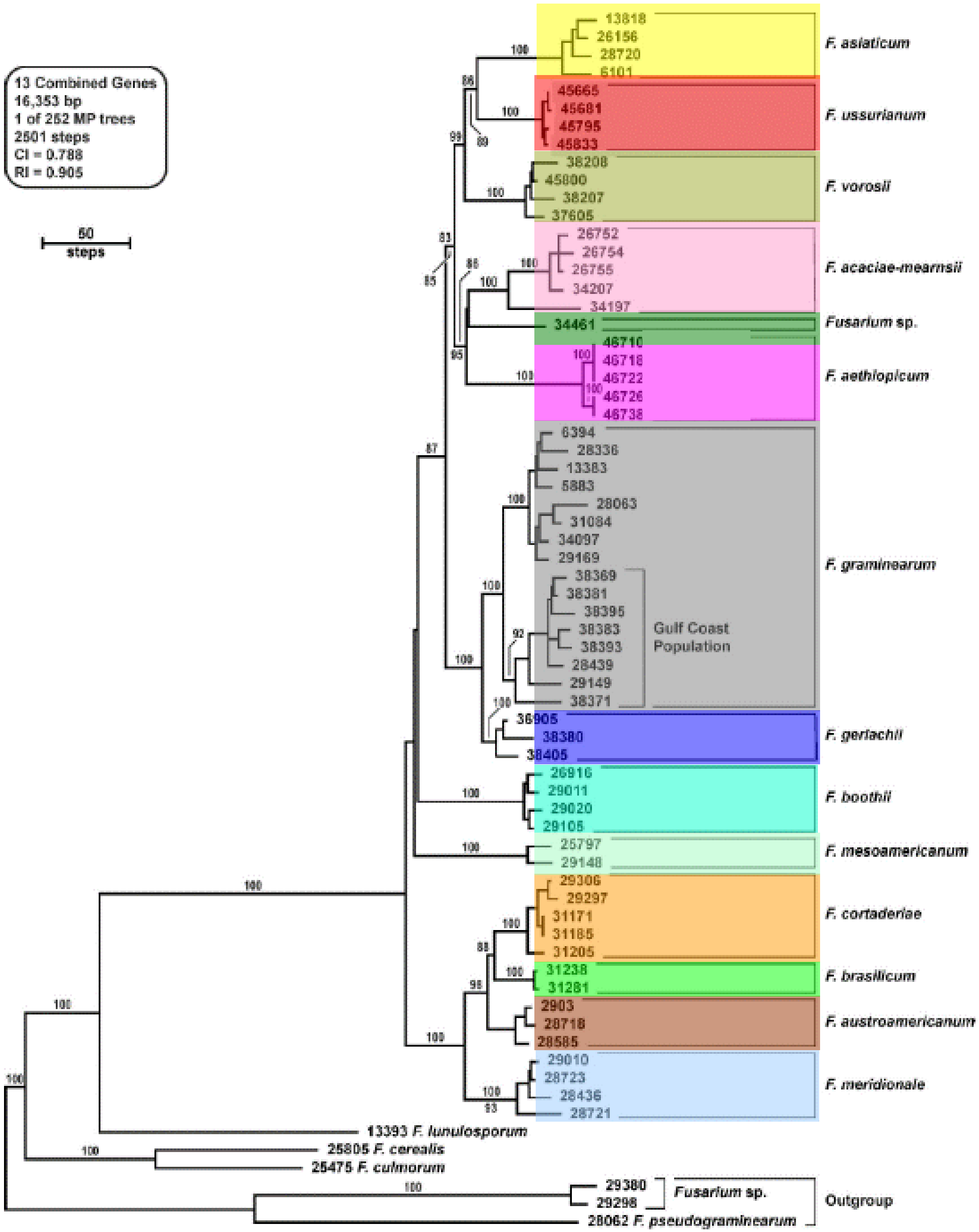


F. graminearum sensu stricto macroconidia

Genotypic differences among isolates of *G. zeae* from field populations have been extensively described.

Recently, it has been proposed that the species is divided into a series of 12 phylogenetic species or lineages on the basis of DNA sequences of 13 combined genes.

The lineages have different geographic distributions, differ in trichotecenes mycotoxins, and may differ in their ability to cause disease on particular crops.

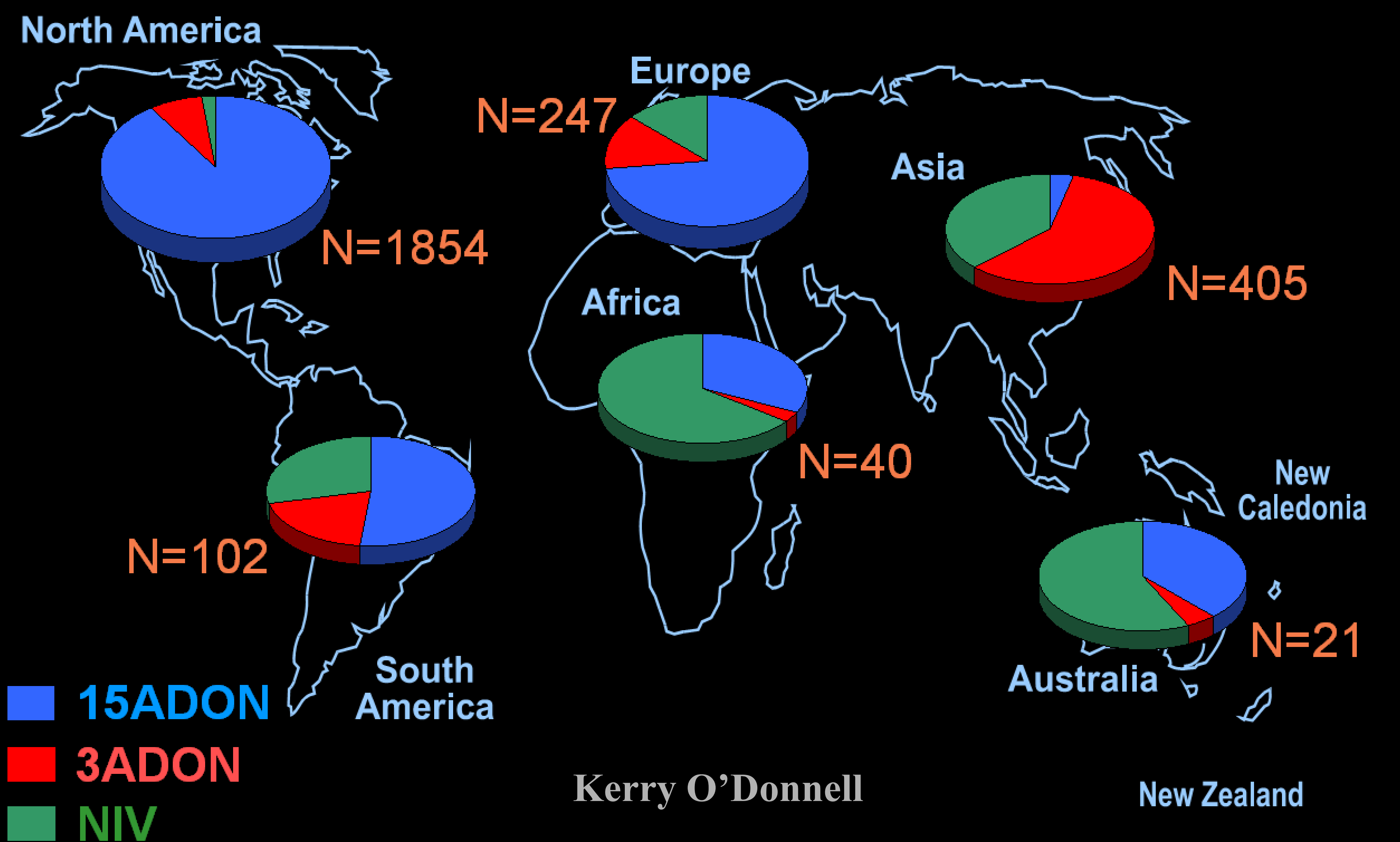


13 genes

…
+
α-tubulin
rDNA

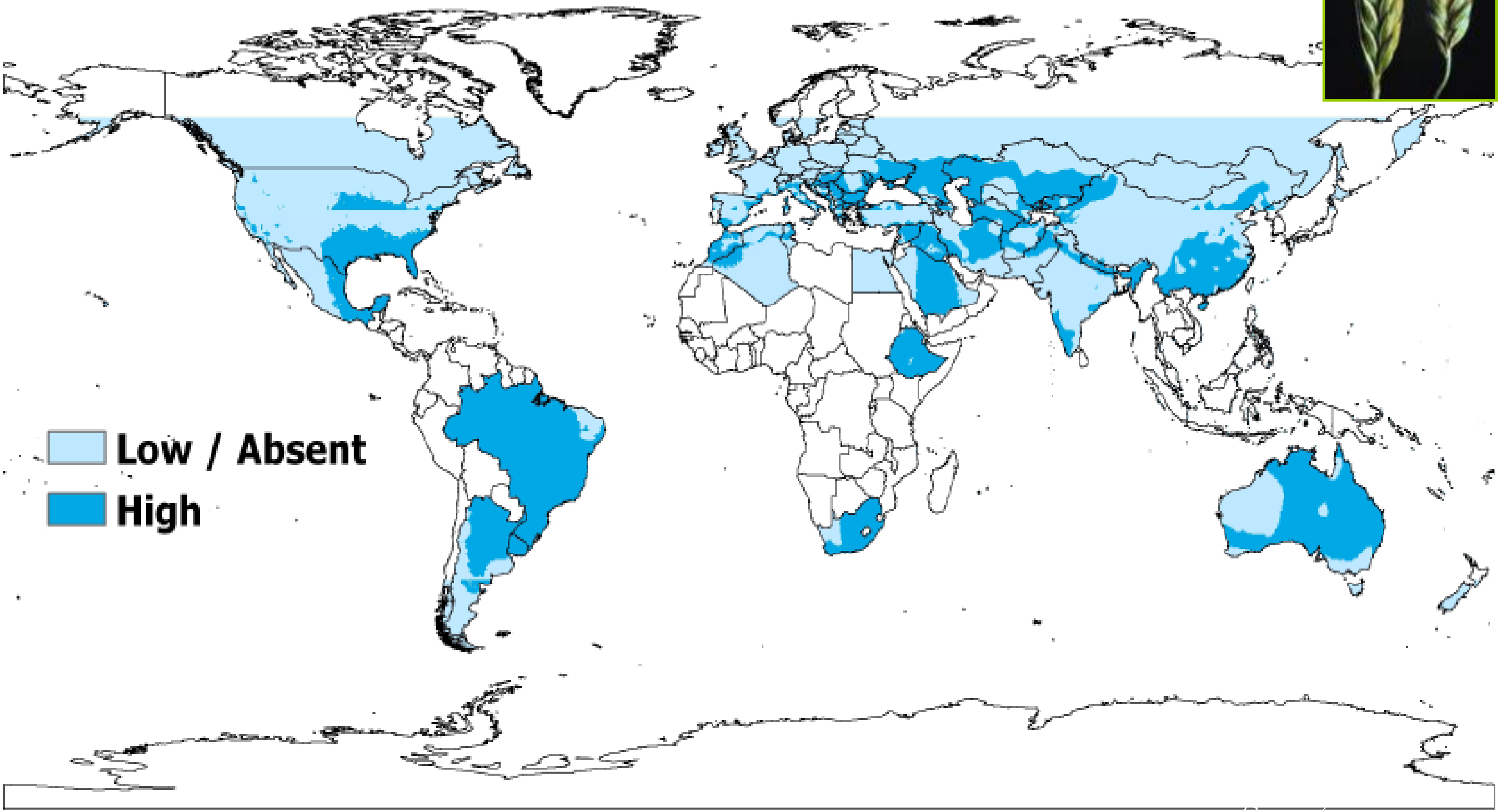
Kerry O'Donnell, 2009

Global Chemotype Distribution



Kerry O'Donnell

Map of DON risk for wheat



Other mycotoxicological risks in cereals

Aspergillus ochraceus

→ OTA ←

Penicillium verrucosum

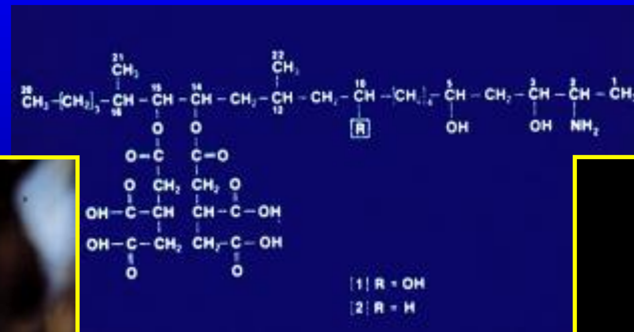
Claviceps purpurea → Alchaloids

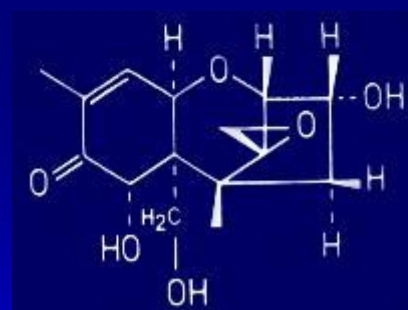
Alternaria alternata → TA, AME

Black point symptoms caused by *Alternaria* spp.

Main species isolated			Main toxins
A. arborescens	sp-grp	:	AOH, AME, altenusin, altenuene
A. tenuissima	sp-grp	:	TeA, ATX-I, AOH, AME
A. infectoria	sp.grp	:	infectopyrone
A. alternata		:	AOH, AME, ATX-I, -II, -III

Fusarium head blight of maize and mycotoxins





Maize ear rot

Maize

Pink ear rot

Fusarium proliferatum
Fusarium verticillioides
Fusarium temperatum
Fusarium subglutinans

Fumonisin-producing species

Red ear rot

Fusarium graminearum
Fusarium culmorum
Fusarium crookwellense

Trichothecene producing species

Aspergillus section Flavi

A. flavus
A. parasiticus
A. minisclerotigenes
A. mottae

Aflatoxigenic species

Aspergillus section Nigri

A. tubingensis
A. welwitschiae (syn A. awamori)
A. niger
A. japonicus

Main *Fusarium* species associated with maize ear rot:

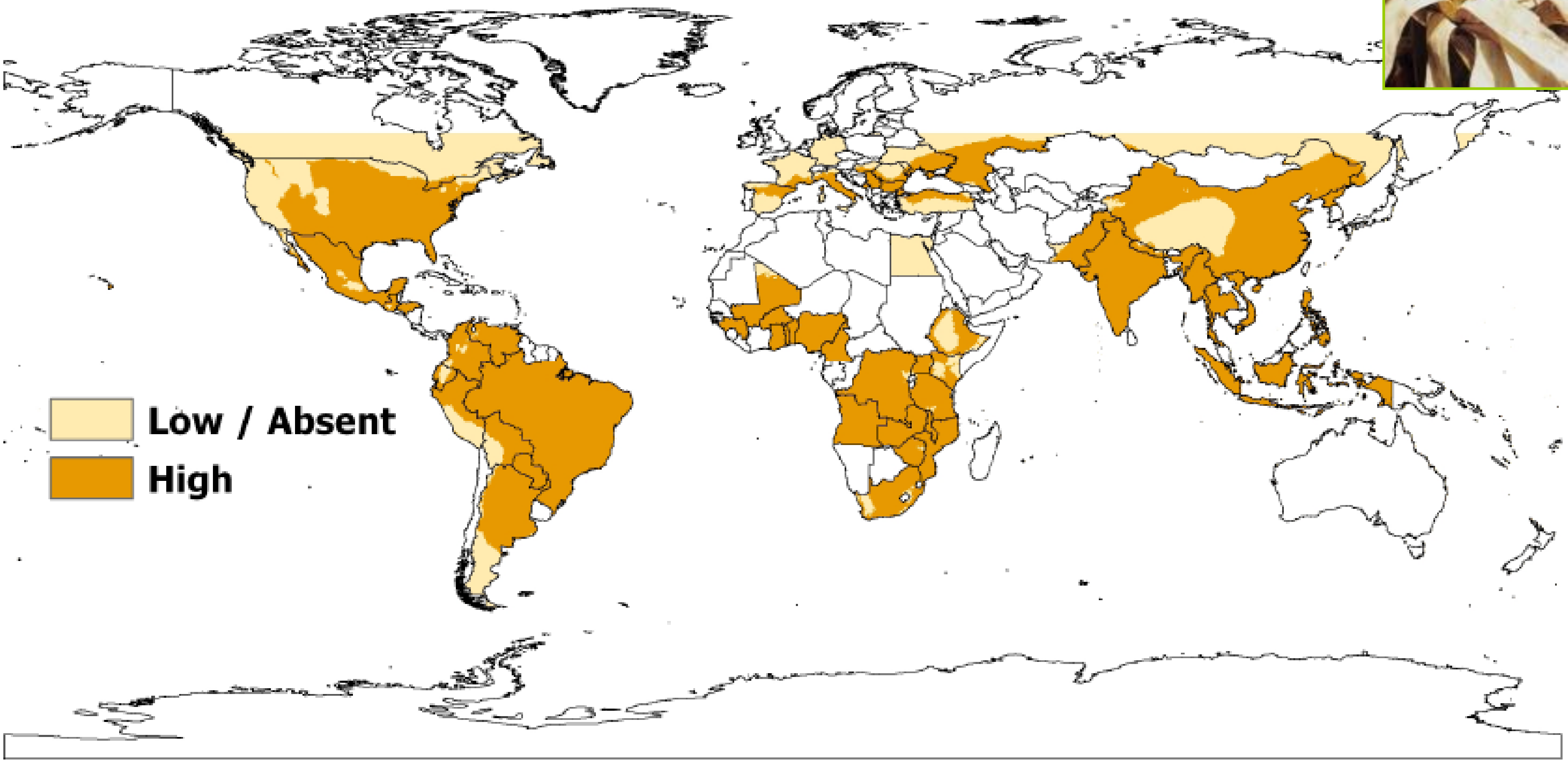
F. verticillioides: FBs (FB1, FB2, FB3)

F. proliferatum: FBs (FB1, FB2)
MON
BEA
FUS

F. subglutinans: MON
BEA
FUS

F. graminearum: DON, NIV, ZEA

Map of Fumonisins risk for maize



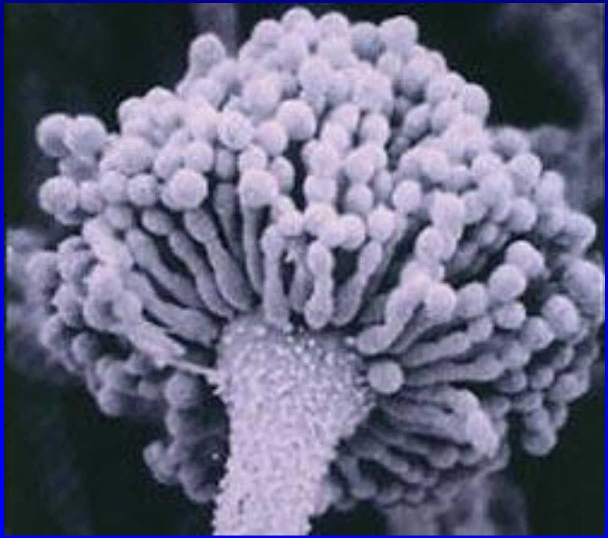
FUMONISIN B_1 OCCURRENCE ON FOOD AND FEED FROM MAIZE

COUNTRIES	FOOD		FEED	
	Samoles	Positive	Samples	Positive
EUROPE	1127	61%	344	79%
AFRICA	367	76%	16	100%
NORTHERN AMERICA	978	47%	684	86%
SUOTHERN AMERICA	234	58%	34	97%
ASIA	878	52%	34	29%
OCEANIA	82	82%	-	-

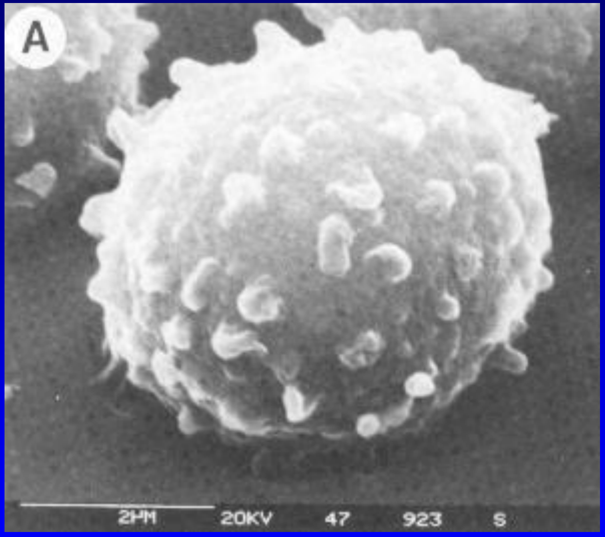
Data from WHO-IPCS Environmental Health Criteria for Fumonisin B_1 (2000)

A.flavus
&
A. parasiticus





A. parasiticus *A.flavus*



Kozakiewicz, 1989

Trade losses due to aflatoxins

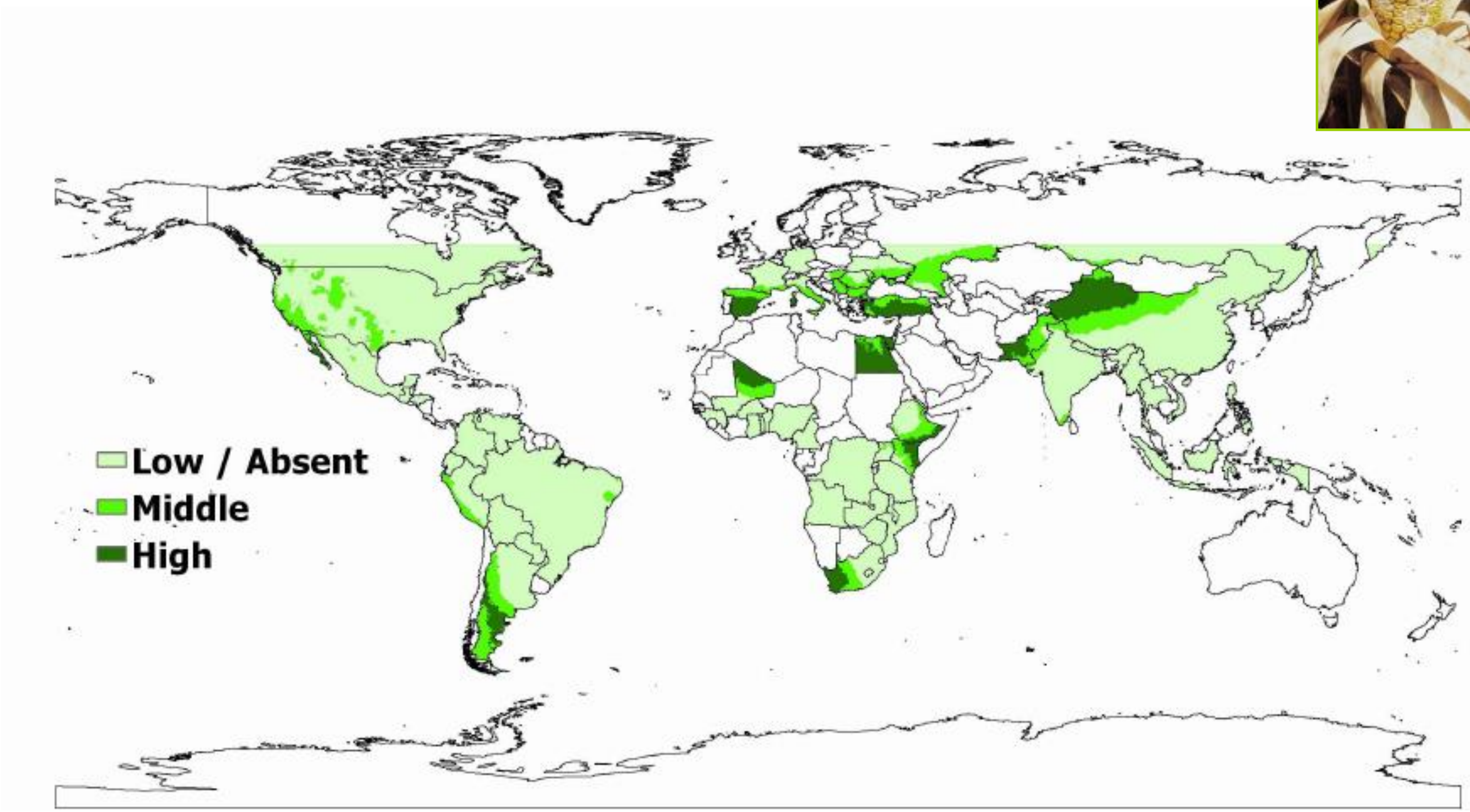
- **Export compliance with food safety and quality standards.**
- **CODEX standard: 20 ppb; EU: 2 ppb**
- **African exports to EU (51%) & the US (22%)**
- **<u>Estimated US$670 million</u> in lost trade for 9 African countries due to EU reduced regulatory levels for aflatoxins**
- **Trade reduction: 46%.**
- **Best quality exported; poorer quality consumed domestically.**

Groundnut

Maize

Mycotoxins

Map of Aflatoxin risk in Maize

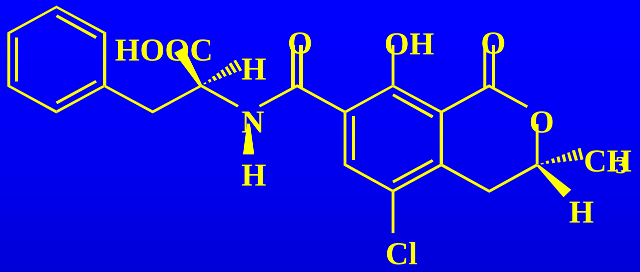


Battilani and Logrieco, 2014

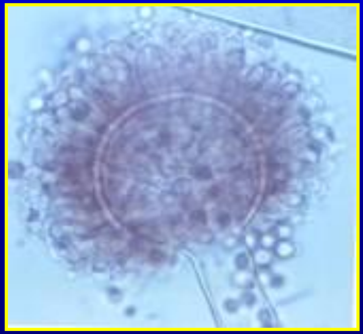
MycoRed

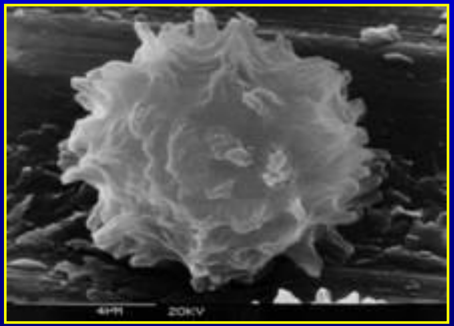
OCHRATOXIN A
IN GRAPE AND WINE

Main ochratoxigenic species isolated from grapes (biseriate)



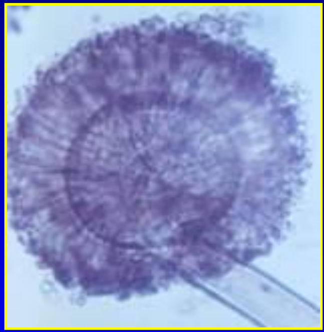
Aspergillus carbonarius

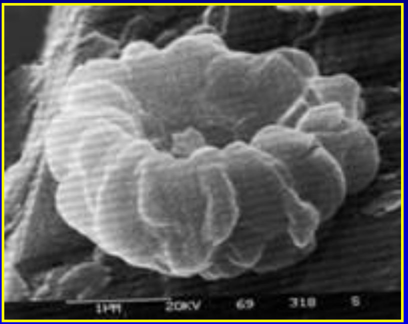




Caracterized by black biseriate conidial head biseriata with big globose and rough conidia

Aspergillus niger aggregate

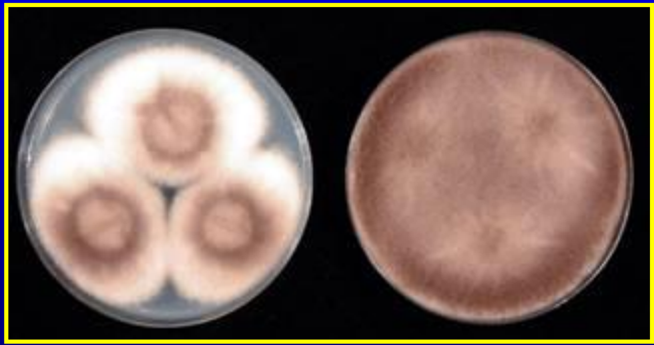


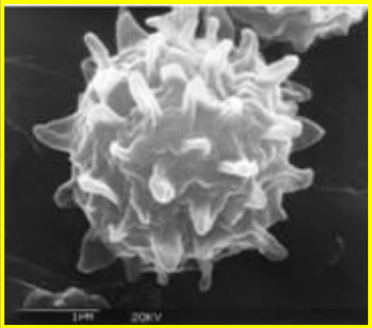


Comprises varius species and/or varietes morphologically indistinguishable and characterize by black-brown conidial head and conidia smallest tha di *A. carbonarius* and variable in shapes and roughness.

Other *Aspergillus* Sez. *Nigri* species isolated from grapes (uniseriate)

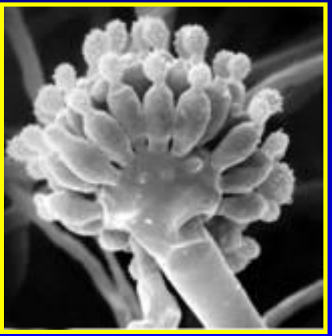
Aspergillus japonicus

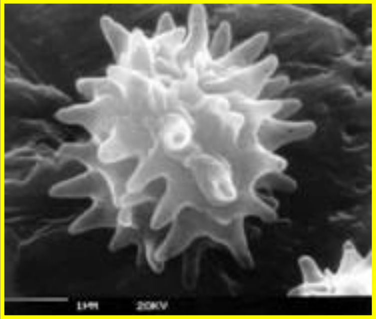




Caracterized by uniseriate conidial head and by globous and spinous conidia

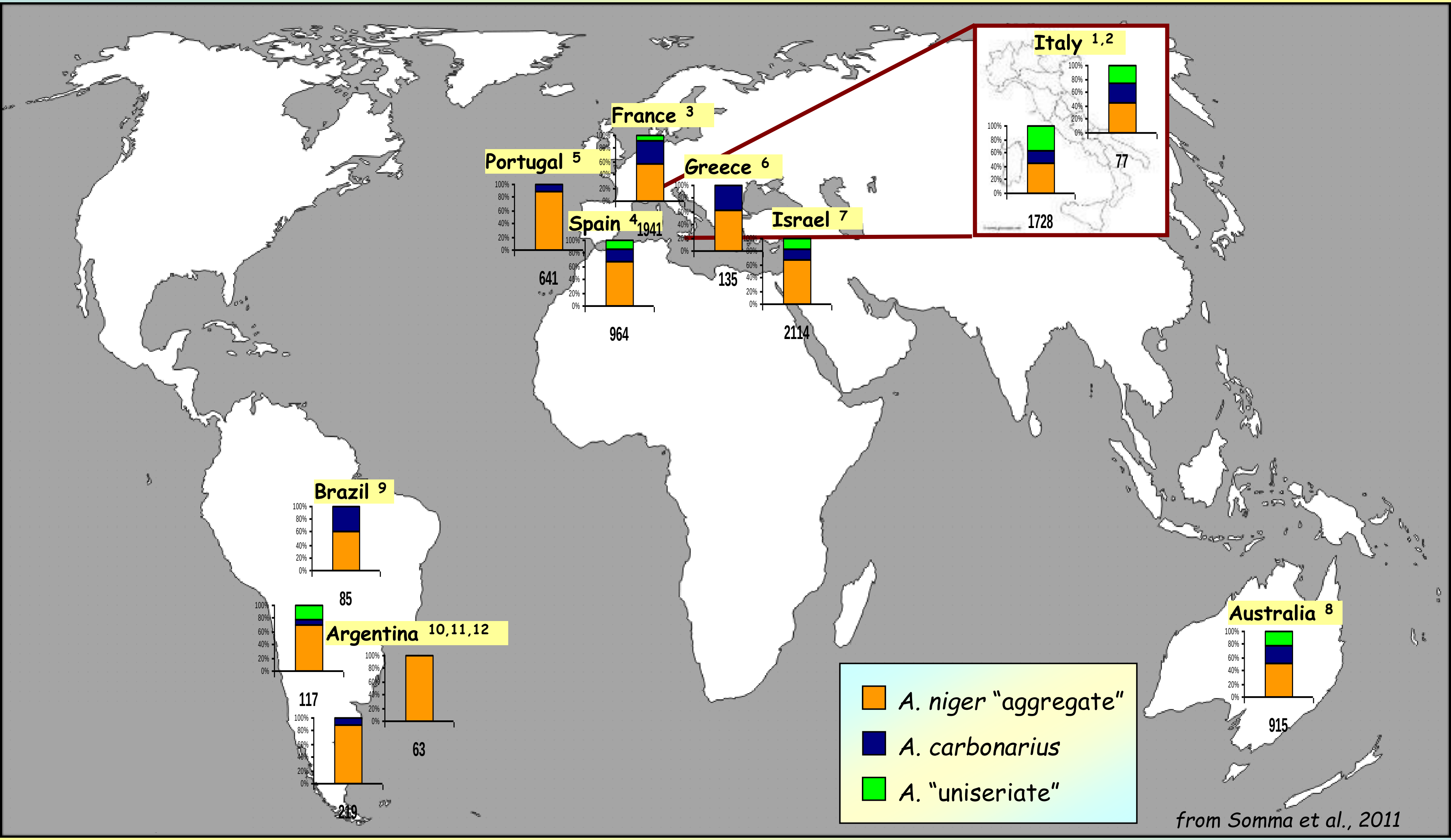
Aspergillus aculeatus





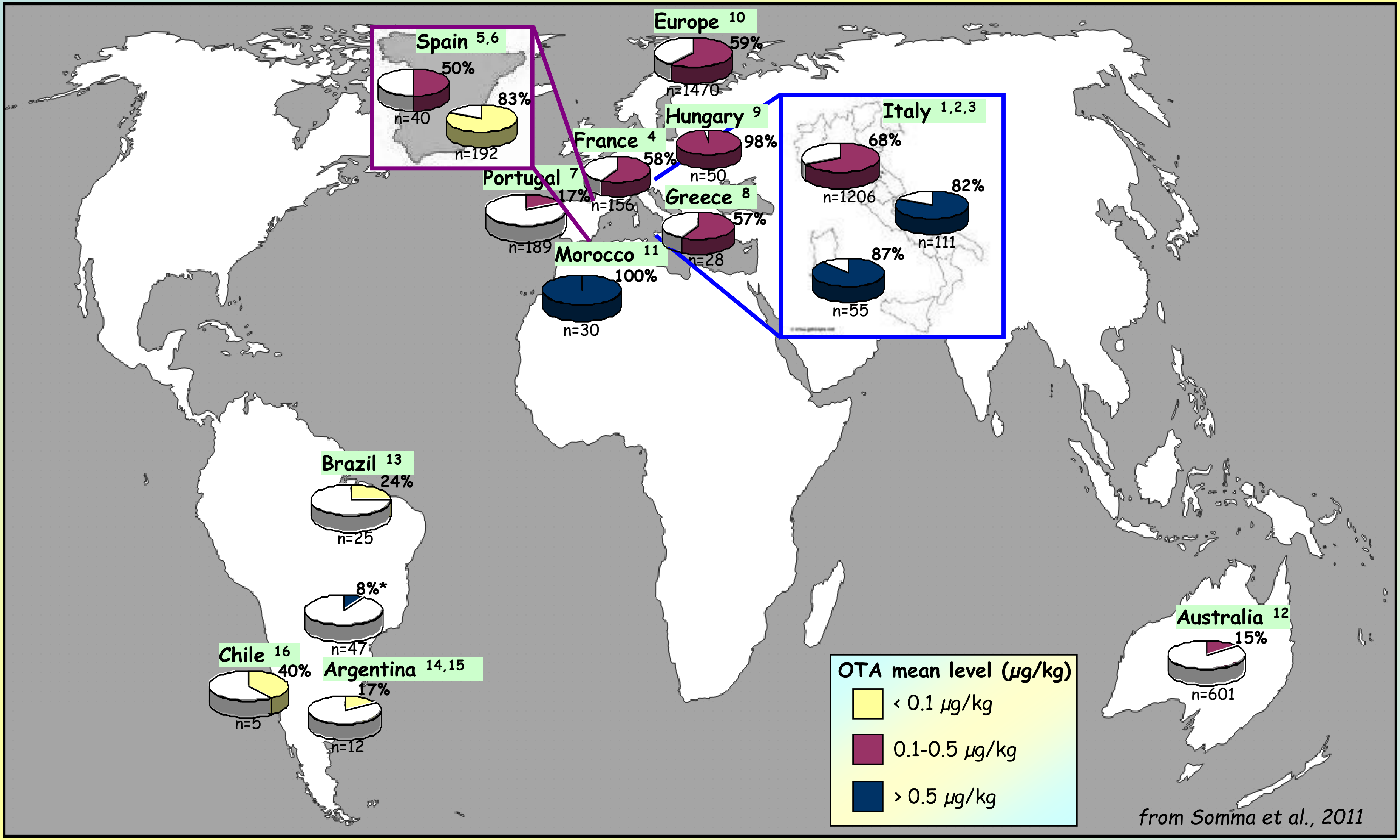
Caracterized by uniseriate conidial head and by echinulated and ellipticus-globous conidial very similar to *A. japonicus.* The two species are morphologically indistinguishable

Distribution worldwide of black *Aspergillus* species on grapes

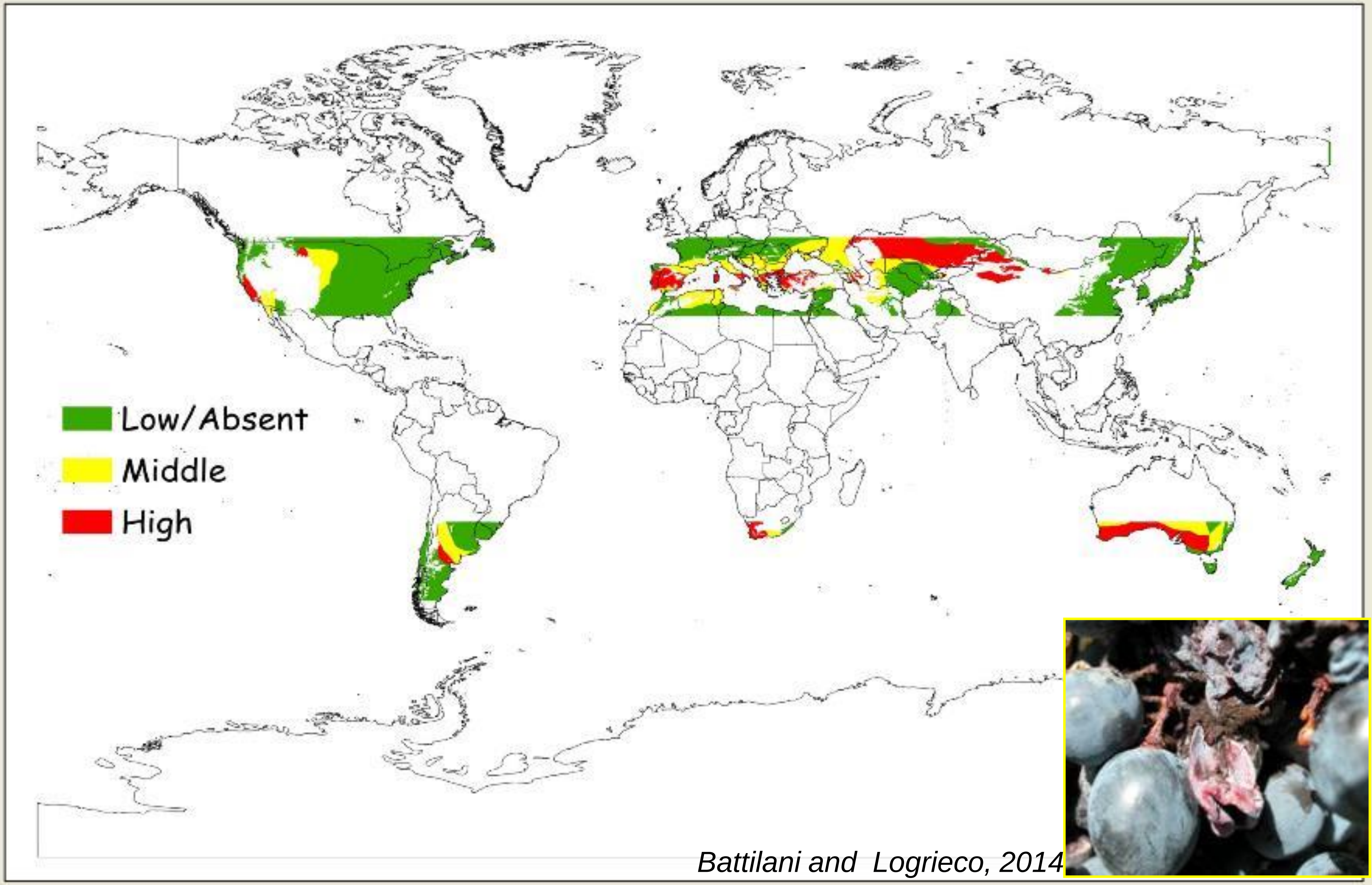


[1] Perrone et al., 2006b
[2] Battilani et al., 2006b
[3] Bejaoui et al., 2006
[4] Belli et al., 2006
[5] Serra et al., 2006
[6] Tjamos et al., 2006
[7] Guzev et al., 2006
[8] Leong et al., 2006a
[9] Da Rocha Rosa et al., 2002
[10] Ponsone et al., 2007
[11] Magnoli et al., 2003
[12] Magnoli et al., 2004

Ochratoxin A contamination in wine

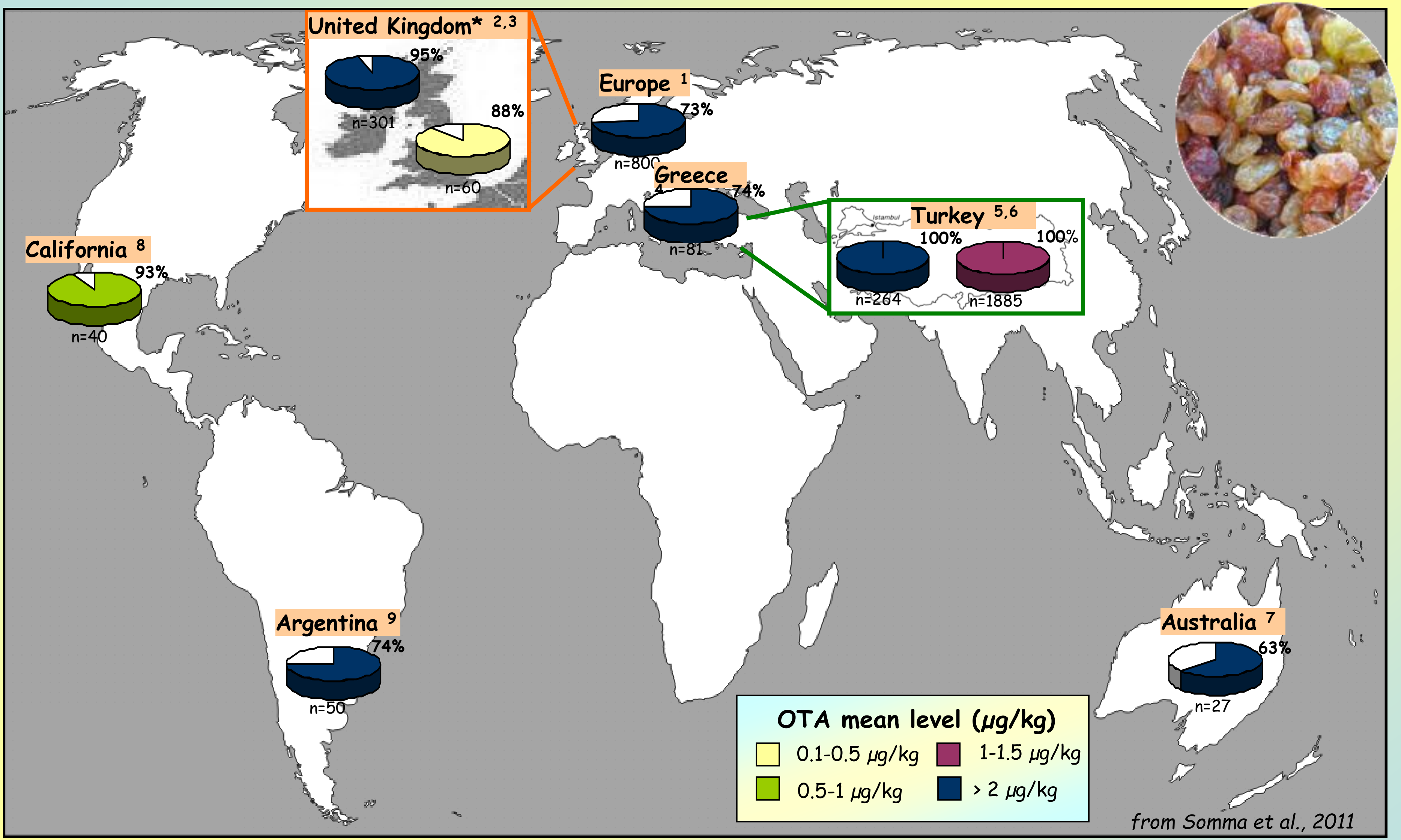


[1] Spadaro et al., 2010
[2] Pietri et al., 2001
[3] Visconti et al., 1999
[4] Clouvel et al., 2008
[5] Lopez de Cerain et al., 2002
[6] Burdaspal and Legarda, 1999
[7] Peito et al., 2004
[8] Soufleros et al., 2003
[9] Varga et al., 2004
[10] Miraglia and Brera, 2002
[11] Zinedine et al., 2010
[12] Hocking et al., 2003
[13] Rosa et al., 2004
[14] Ponsone et al., 2010
[15] Rosa et al., 2004
[16] Rosa et al., 2004



Prediction map of ochratoxin A risk in grapes growing areas on a global scale

Ochratoxin A contamination in dried vine fruits



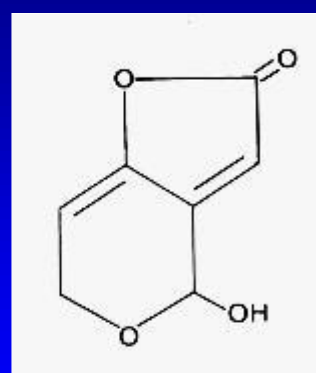
[1] Miraglia and Brera, 2002
[2] MAFF, 1999
[3] Mac Donald et al., 1999
[4] Stefanaki et al., 2003
[5] Meyvaci et al., 2005
[6] Aksoy et al., 2007
[7] Leong et al., 2006
[8] Palumbo et al., 2011
[9] Magnoli et al., 2004

* The United Kingdom data are based on market samples.

Patulin in apples & pears

Fruit juice

Penicillium expansum



Patulin

Natural occurrence of *Alternaria* toxins on mandarin fruit

Black: Tenuazonic acid (21-87 ppm)

Alternariol methyl ether (0.5-1.4 ppm)

Alternariol (1-5.2 ppm)

Gray: Tenuazonic acid (173 ppm)

Toxigenic fungi associated with figs :

Aspergilus flavus

Aspergillus niger & A. carbonarius

Fusarium ramigerum

F. lactis

F. proliferatum

Natural occurrence of toxins associated with fig endosepsis:

Aflatoxins

Ochratoxin A

Fumonisins (FBs)

Fusaproliferin (FUS)

Pepper & Tomato

SPECIES
Alternaria alternata

Natural occurrence of *Alternaria* toxins on pepper and tomato:

Alternariol (7,200 ng/g)

Alternariol methyl ether (270 μg/g)

Tenuazonic acid (1,300 μg/g)

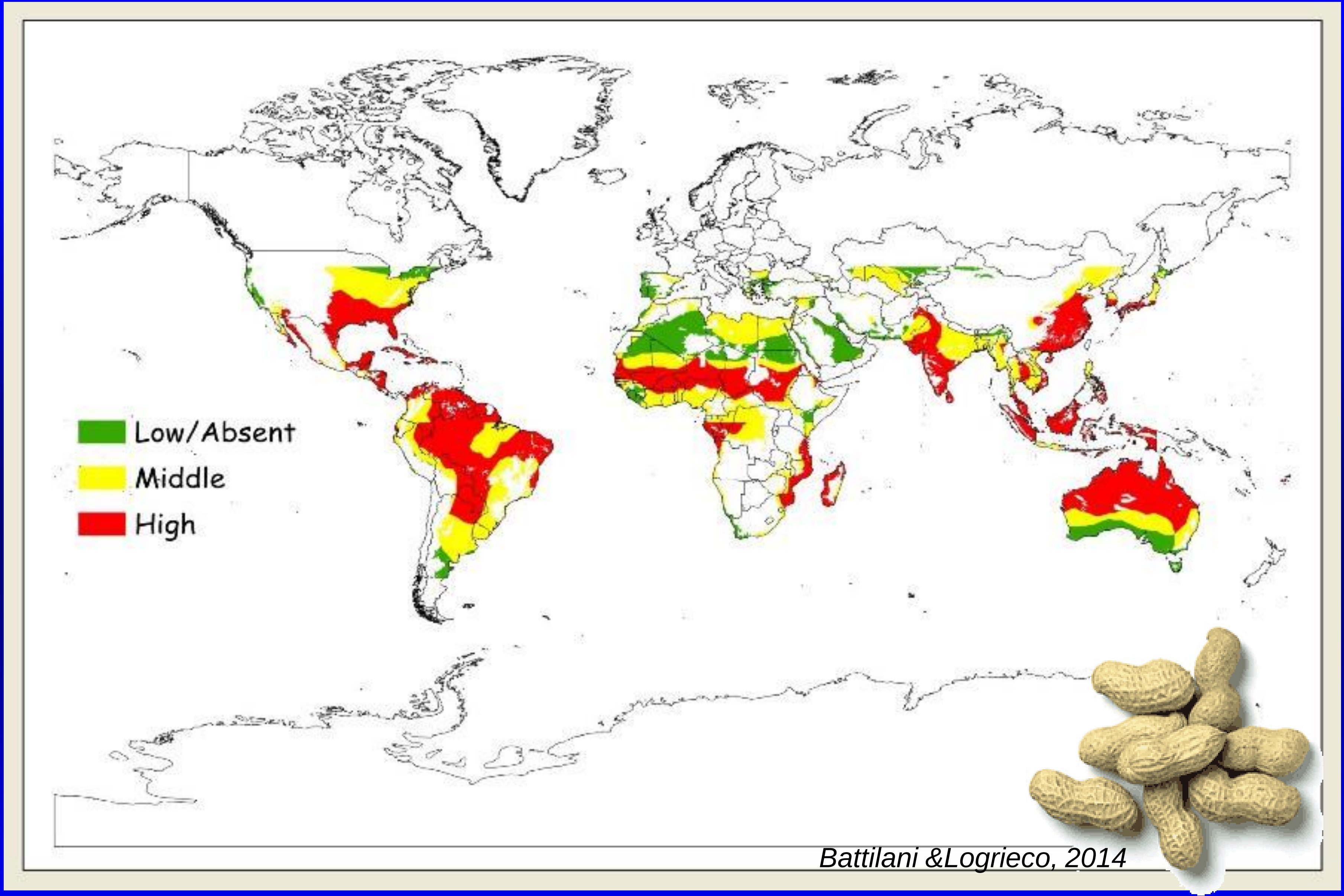
Dried fruits

Dominant fungal species
occurring in dried fruits
(apricots, dates and prunes):

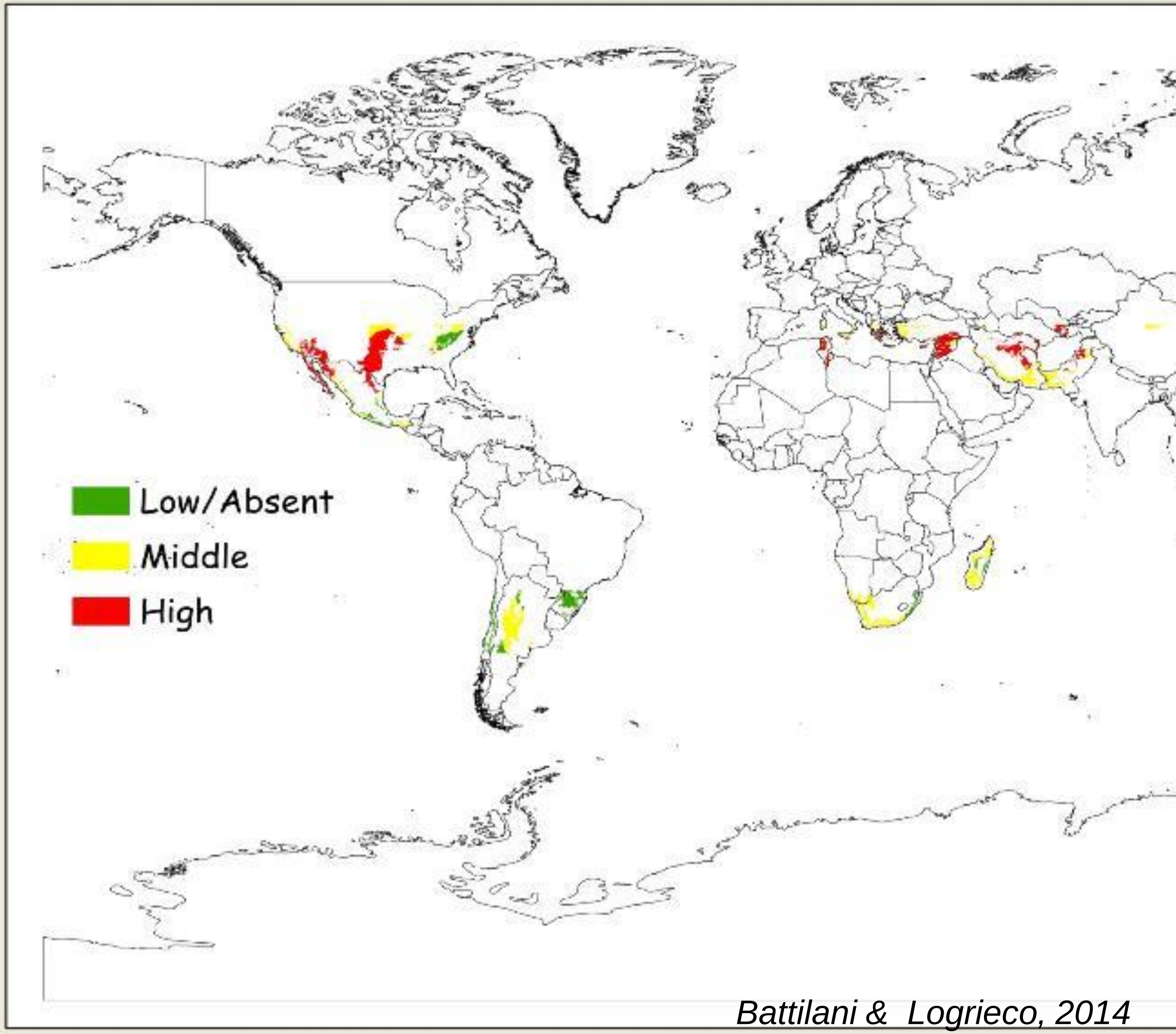
Aspergillus and *Penicillium*

The most important mycotoxins
occurring in these Mediterranean
products:

Aflatoxins (B1, B2, G1 and G2)
and **OTA**



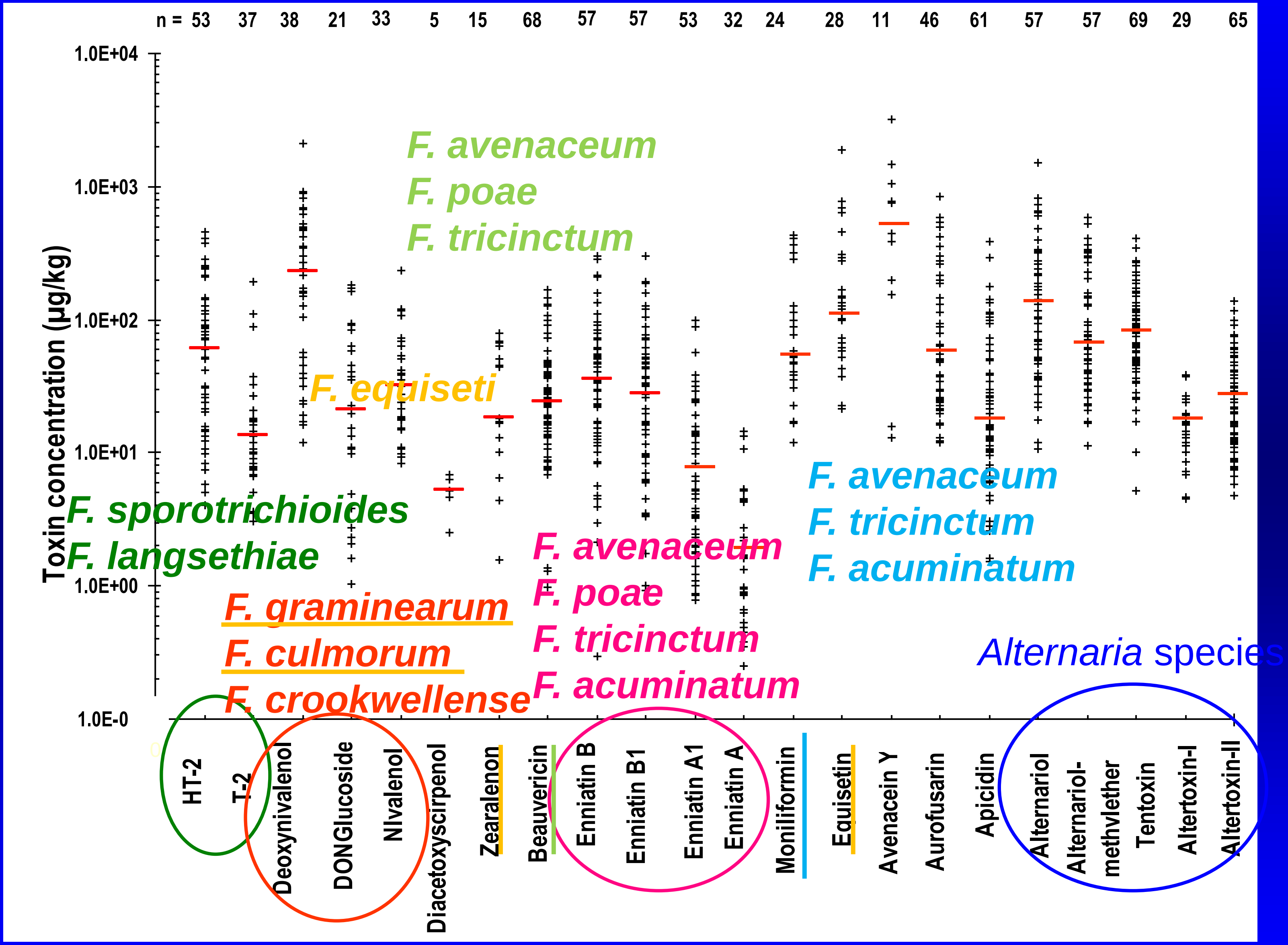
Prodiction map of <u>aflatoxin B_1</u> risk in <u>peanuts</u> growing areas on a global scale



Battilani & Logrieco, 2014

Prediction map of aflatoxin B_1 risk in pistachios growing areas on a global scale

Multitoxin analysis in cereal samples



Fungal metabolite prevalence in 71 naturally contaminated cereal samples

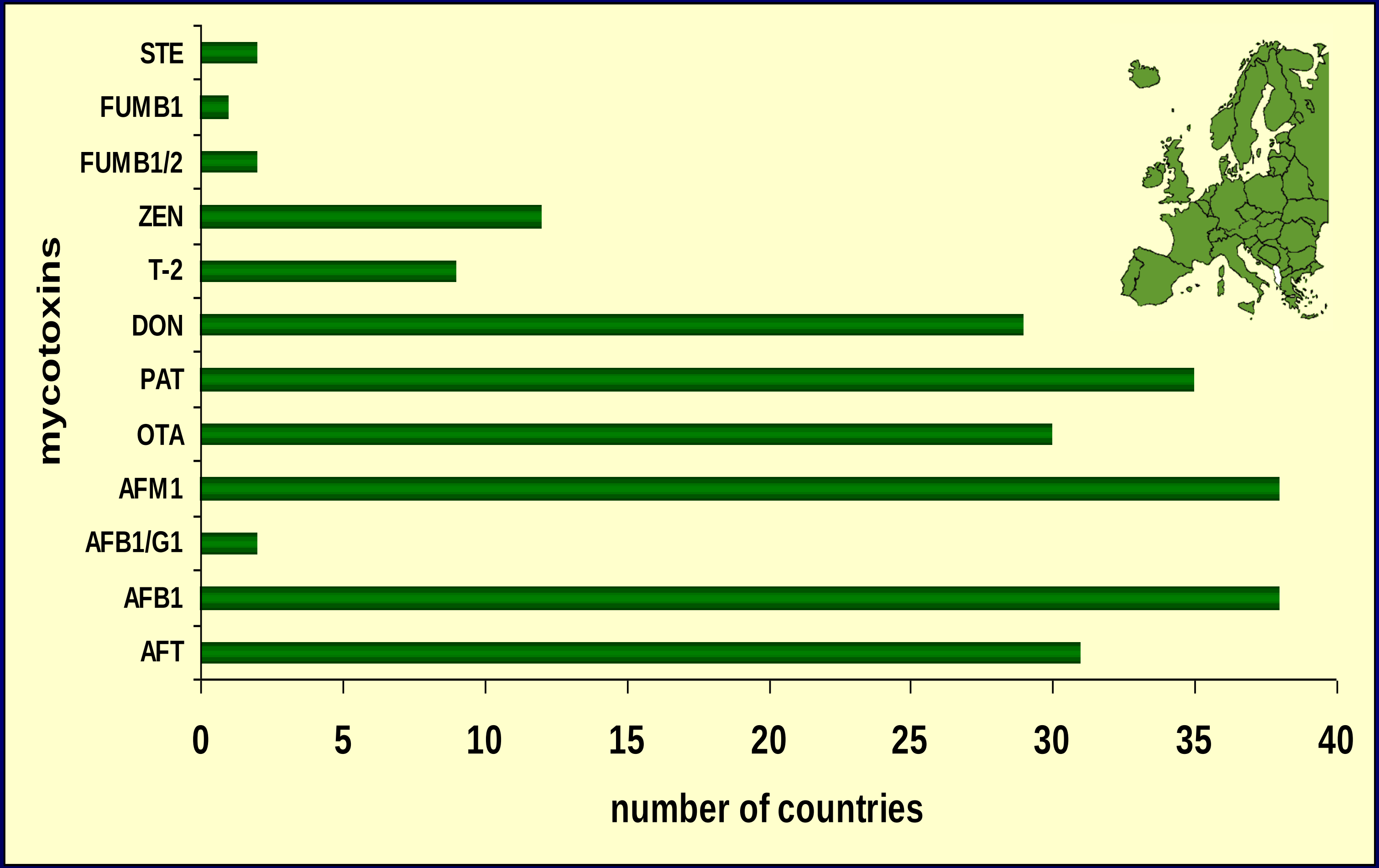
Mycotoxin regulating countries in Europe

(FAO FNP 81, 2004)

- **39 nations** with known regulations (99 % of inhabitants of the region)
- **EU harmonized limits** exist for **aflatoxins**, **ochratoxin A**, **patulin**, **DON**, **zearalenone**, **fumonisins**
- **EU food limits considered** for T-2/HT-2, ergot alkaloids and other mycotoxins
- **EU feed limits** exist for aflatoxin B_1
- **EU feed guidance** values exist for ochratoxin A and some *Fusarium* toxins

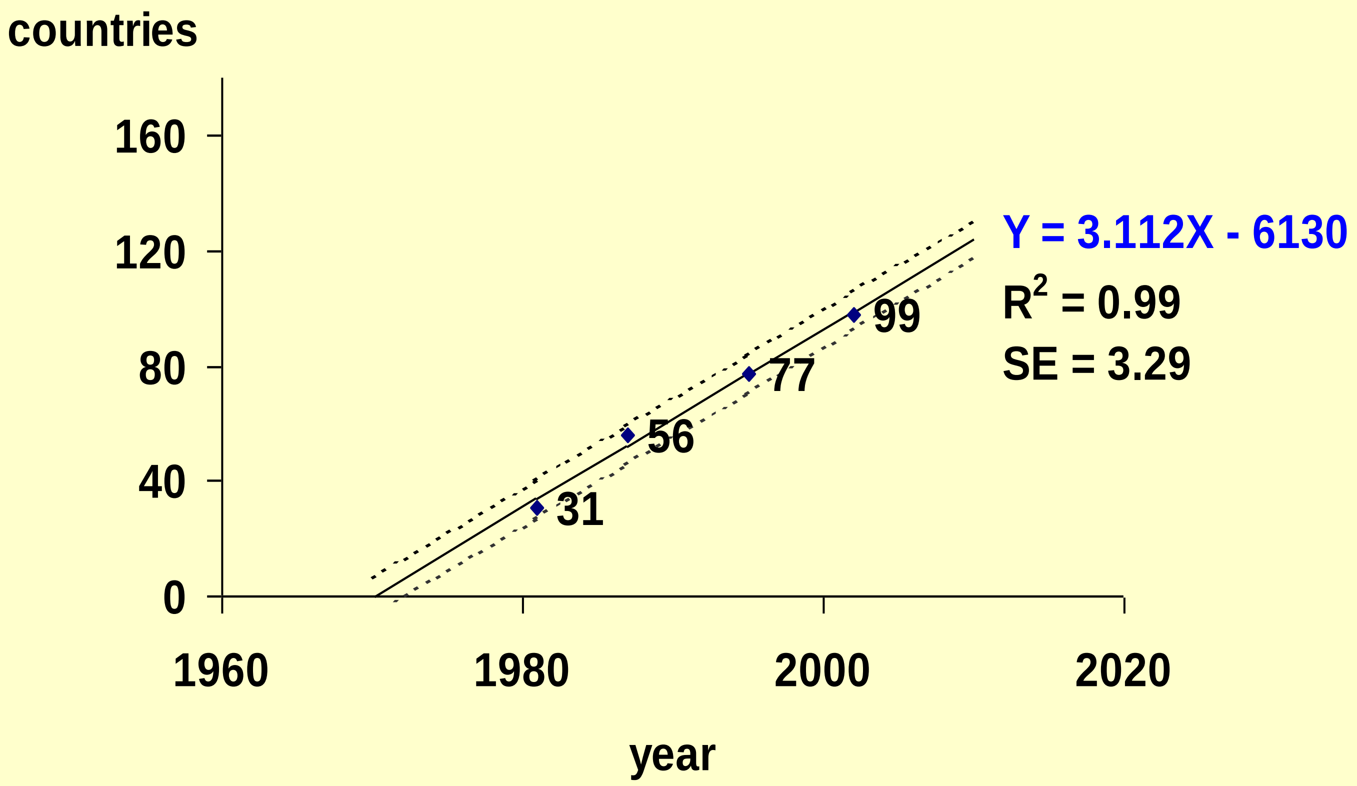
Europe: mycotoxins regulated in food

(FAO FNP 81, 2004)

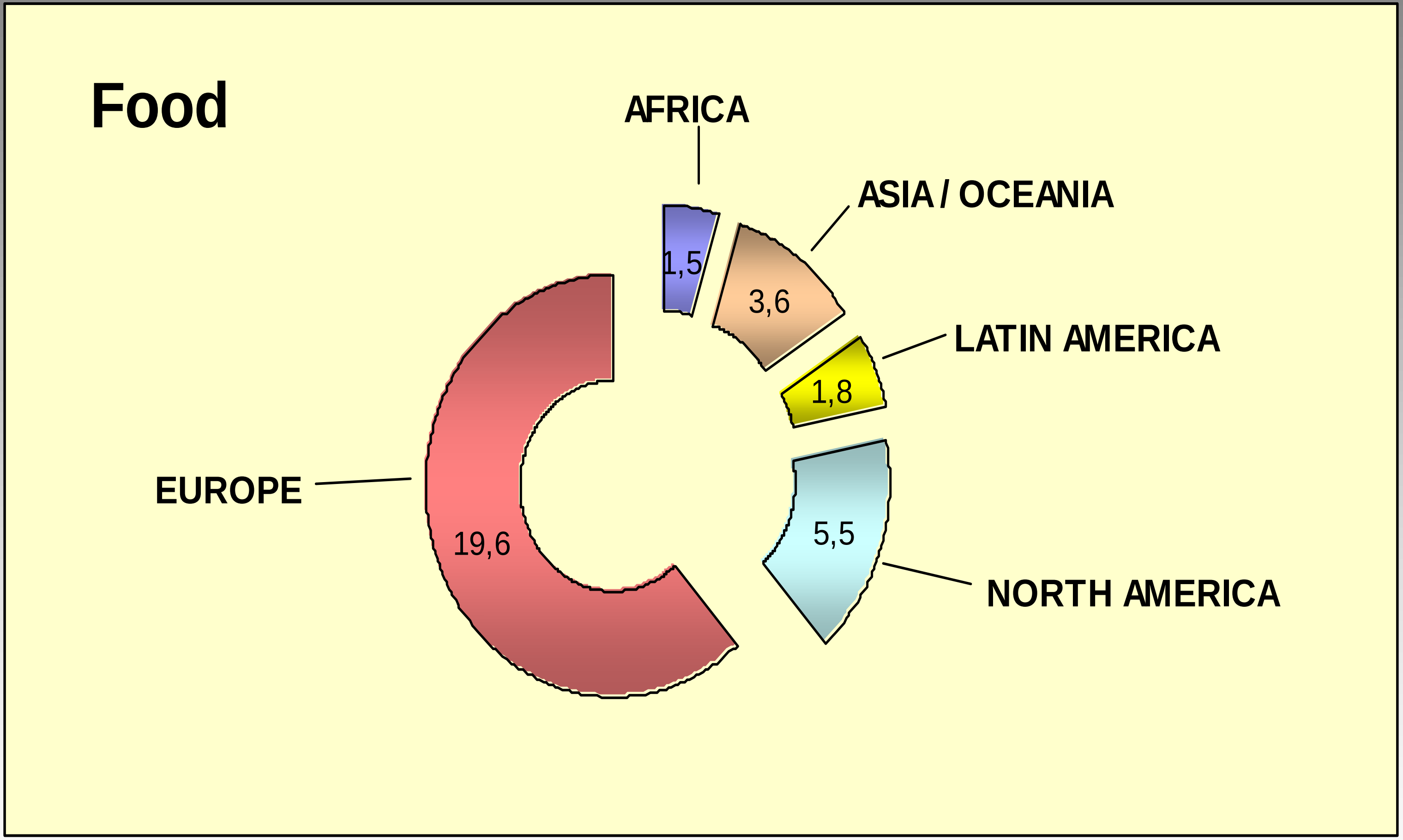


Mycotoxin - regulating countries

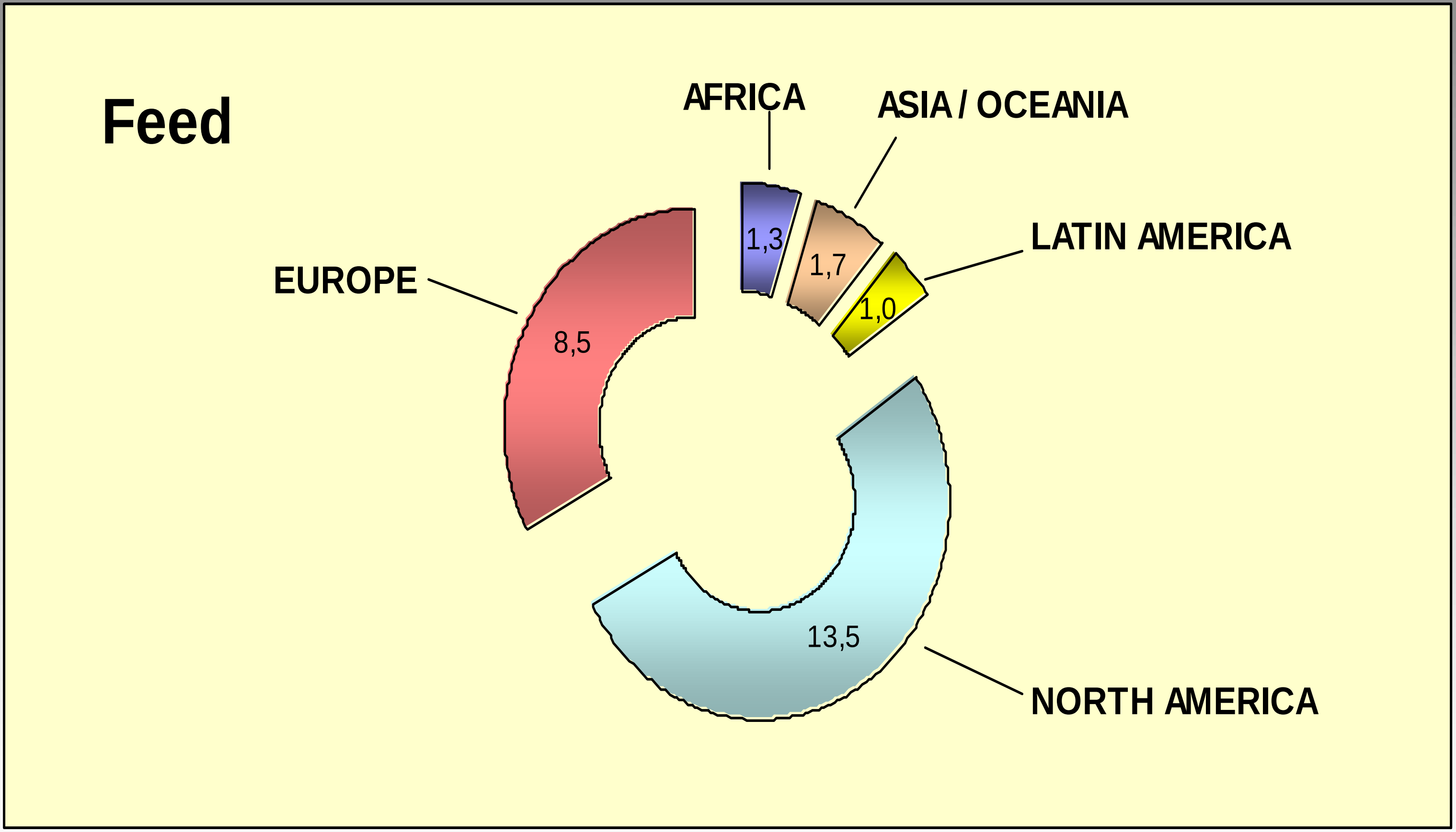
1981 - 1987 - 1995 - 2003



Number of mycotoxin regulations per country



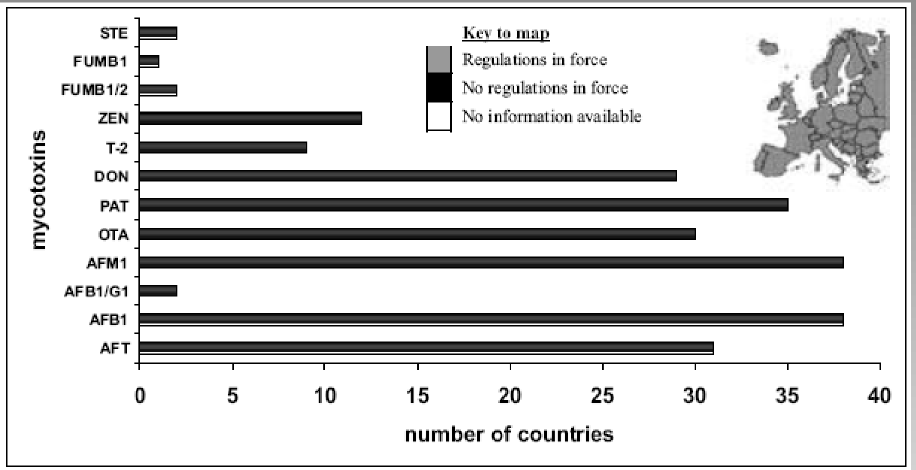
Number of mycotoxin regulations per country

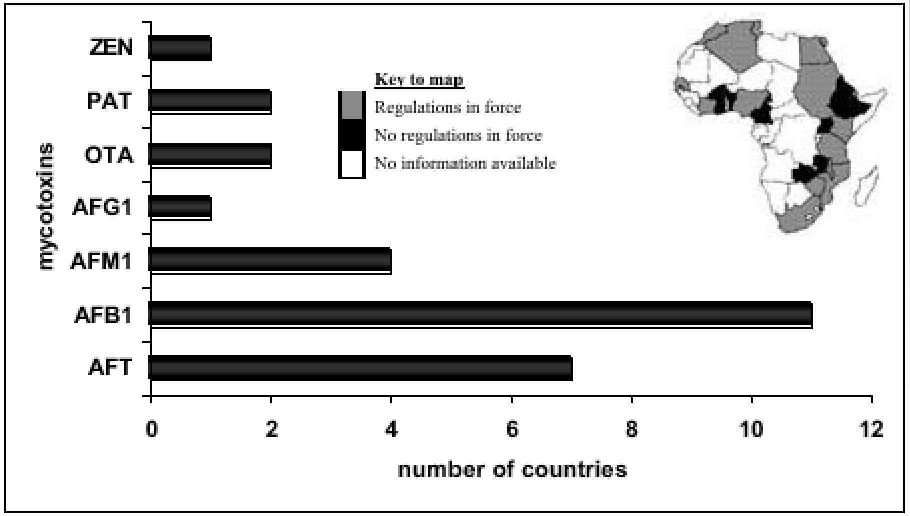


Mycotoxin regulation in Europe & Africa

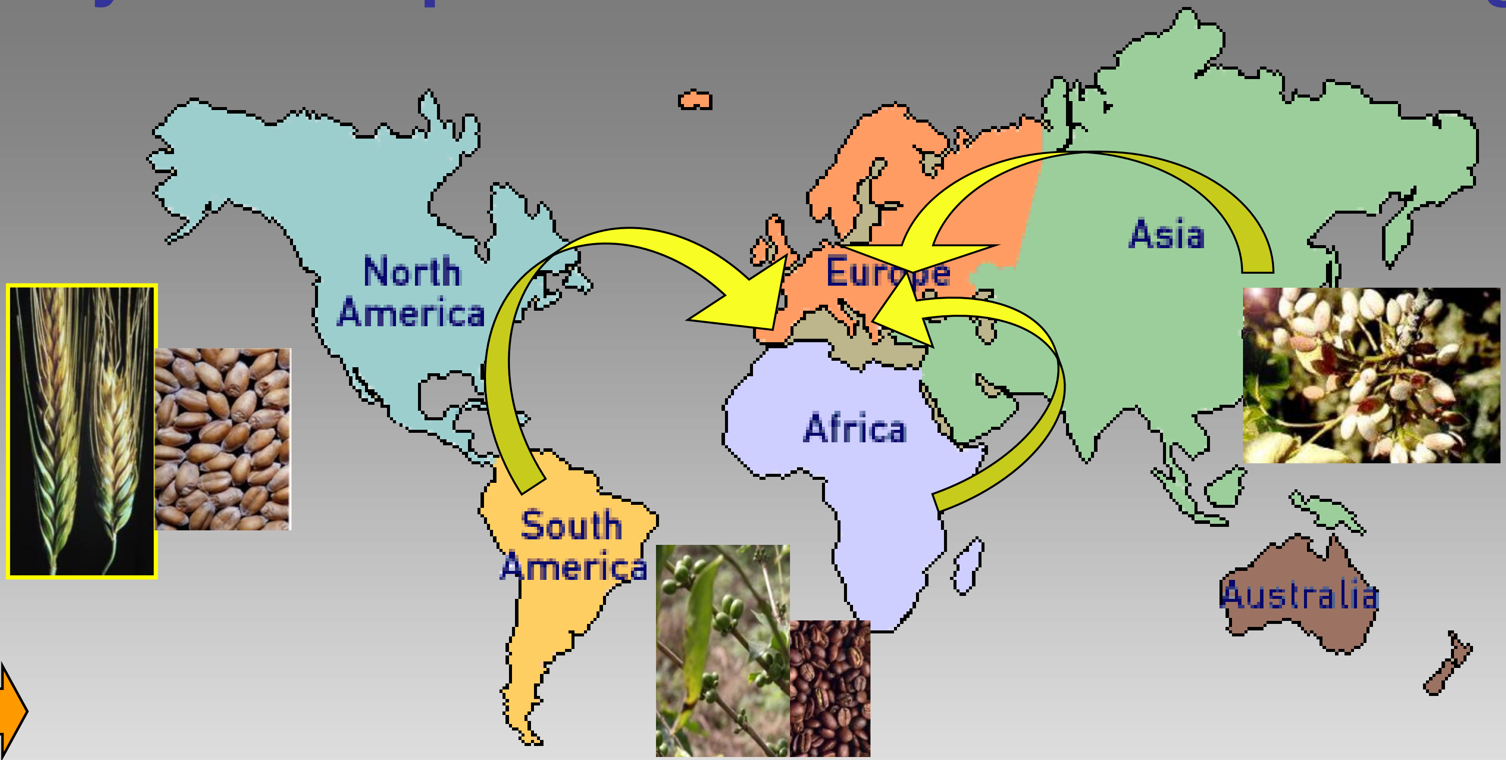
Source: FAO, 2004

- All EU member states have regulations for 12 mycotoxins
- 15 African countries have regulations (59% pop).
- Most countries not regulated, but say that regulations needed
- Does not mean problem does not exist
- For small-scale & subsistence systems regulation has failed





Mycotoxin problems due to trade exchanges



Imported products with high risk of mycotoxin contamination:

- **maize**, **(fumonisins and aflatoxins) from all continents**
- **cereals (deoxynivalenol, ochratoxin A) mostly from north and south America**
- **coffee, (ochratoxin A) mostly South America & Africa**
- **pistachio nuts, (aflatoxins) mostly from North Africa & Asia**
- **Peanuts & other nuts, (aflatoxins) mostly North, South America & Africa**
- **Spices (aflatoxins) mostly from Asia & Africa**

2012-2015 notifications by hazard category

	2012	2013	2014	2015
	82	164	89	95
	85	70	78	137
	43	51	37	44
	16	25	20	18
	2	3	4	8
	189	166	200	109
	34	13	1	
	138	91	130	140
	155	92	93	107
	89	76	51	45
	238	272	275	208
	37	33	64	42
orrect	43	10	12	26
Migration	167	85	93	77
Mycotoxins	446	368	357	476
Non-pathogenic microorganisms	86	32	37	47
Not determined/other	11	15	8	11
Organoleptic aspects	79	36	39	38
Packaging defective/incorrect	34	20	24	17
Parasitic infestation	55	10	18	11
Pathogenic microorganisms	458	643	630	637
Pesticide residues	436	450	430	398
Poor or insufficient controls	137	94	58	87
Radiation	50	20	12	26
Residues of veterinary medicinal products	54	86	95	56
TSEs	5	2		

Rapid Alert System for Food and Feed

2016 notifications by hazard category and by classification

hazard category	*alert*	*border rejection*	*information for attention*	*information for follow-up*
adulteration / fraud		107	1	4
allergens	87	4	16	6
biocontaminants	18	6	22	
biotoxins (other)	12		6	1
chemical contamination (other)			1	1
composition	93	15	35	36
feed additives	1			2
food additives and flavourings	41	62	43	22
foreign bodies	76	14	10	34
GMO / novel food	12	11	18	52
heavy metals	78	57	71	12
industrial contaminants	23	14	19	5
labelling absent/incomplete/incorrect	8	5	7	8
migration	9	40	18	11
mycotoxins	82	418	49	2
non-pathogenic micro-organisms	2	22	8	30
not determined / other	4	3	2	1
organoleptic aspects	2	27	3	8
packaging defective / incorrect	5	15	1	4
parasitic infestation		3	11	9
pathogenic micro-organisms	250	159	183	93
pesticide residues	38	142	62	11
poor or insufficient controls	2	78	4	13
radiation		1	2	2
residues of veterinary medicinal products	10	12	12	13
TSEs			3	5

Rapid Alert System for Food and Feed

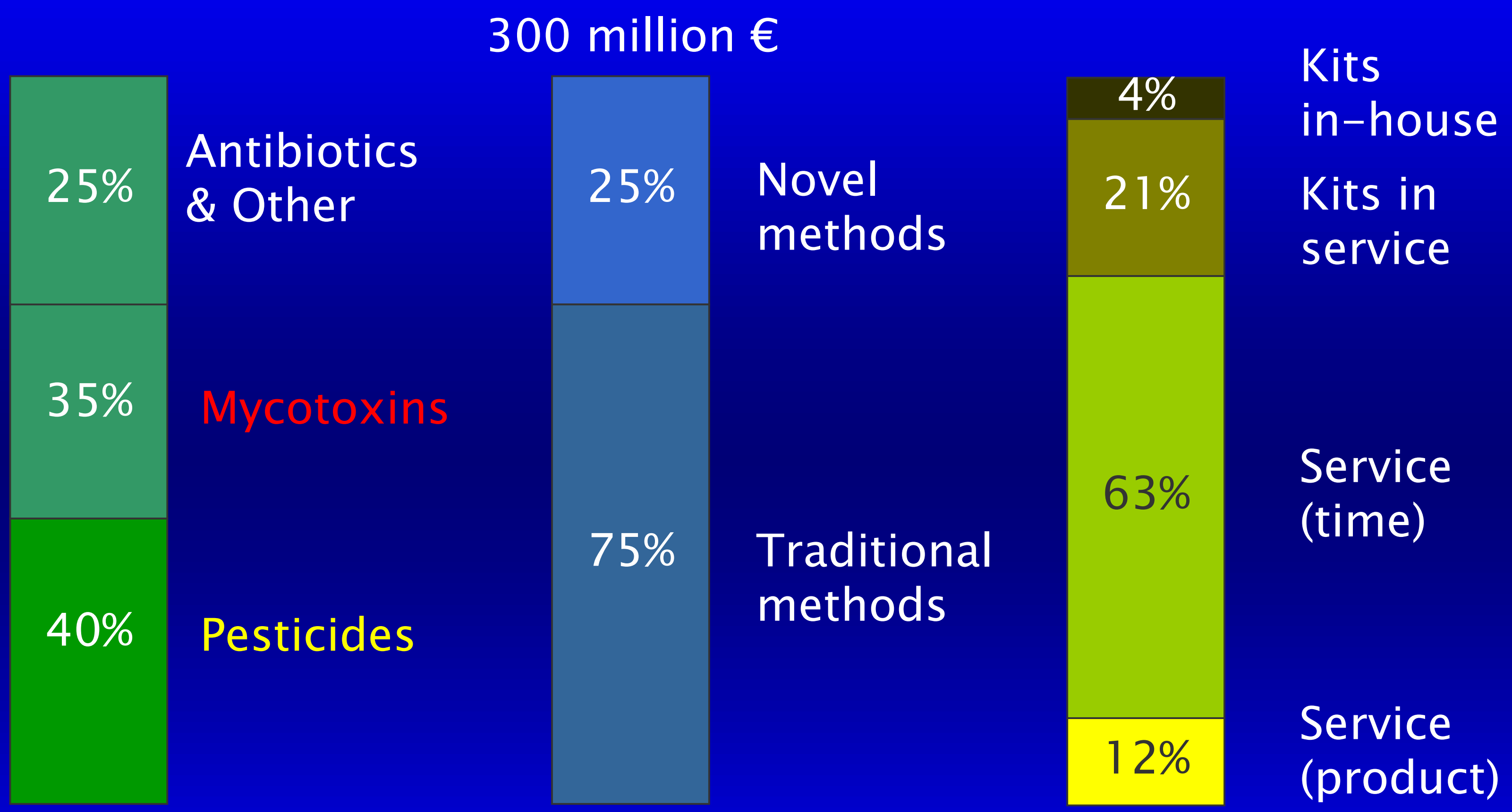
2016 - top 10 number of notifications

Number of notifications counted for each combination of hazard/product category/country.

- by origin

hazard	product category	origin	notifications
pesticide residues	fruits and vegetables	Turkey	77
aflatoxins	nuts, nut products and seeds	Turkey	68
mercury	fish and fish products	Spain	62
aflatoxins	nuts, nut products and seeds	Iran	56
aflatoxins	nuts, nut products and seeds	China	49
aflatoxins	nuts, nut products and seeds	United States	45
Salmonella	fruits and vegetables	India	46
aflatoxins	fruits and vegetables	Turkey	40
aflatoxins	nuts, nut products and seeds	Egypt	32
aflatoxins	herbs and spices	India	32

Global diagnostics market
Chemical contaminants

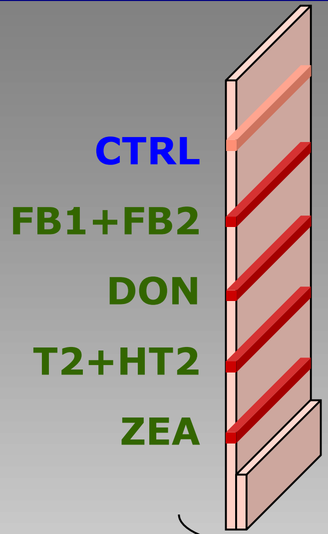


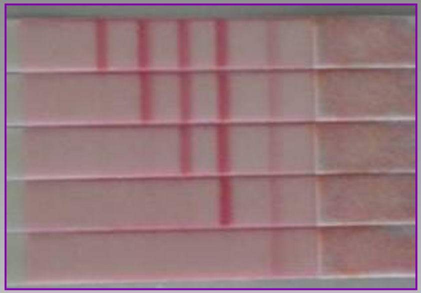
- estimated 55 million tests
- Average cost of test (ACT): 50-60€ (1.5€ ÷100 €)
- Expected annual growth to 2020: volume 6%, value10-12%

Multiplex dipstick - *Fusarium* toxins in cereals, cereal food, maize feed

wheat/oats/maize
maize feed
breakfast cereals

Methanol/water extraction





Negative sample
Positive ZEA
Positive ZEA/T2
Positive ZEA/T2/DON
Positive ZEA/T2/DON/FB1

Dilution with buffer

Incubation at 40°C, 10 min
Migration, 10 min

Dipstick reader
(*Readsensor*)

Total time of analysis: 30 min

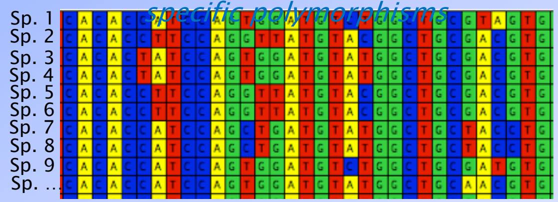
Lattanzio et al., Analitica Chimica Acta 2012, 718:99-108

ISTITUTO DELLE PRODUZIONI ALIMENTARI

Character-based barcodes as a species diagnostic tool

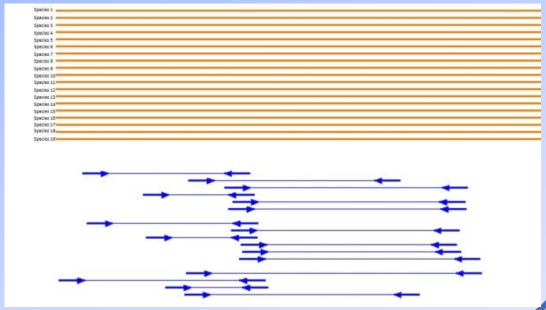
appropriate gene target for PCR assays

A single locus for fungal species occurring on maize , including species-specific polymorphisms



species-specific primers design

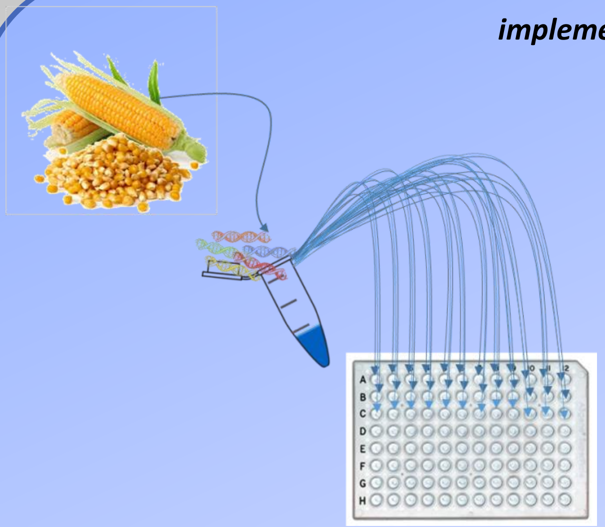
*Various prime pairs to identify different species or group of species belonging to **Fusarium, Aspergillus** and **Penicillium** genera*

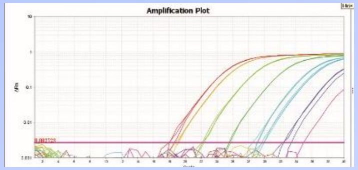


FP6-IST-1-508774-IP

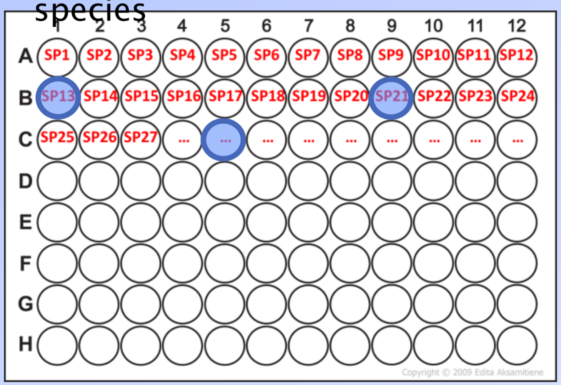
Development of **array architecture** for the **detection** of fungal contaminants on crop sample

implementation of assays in a PCR Array





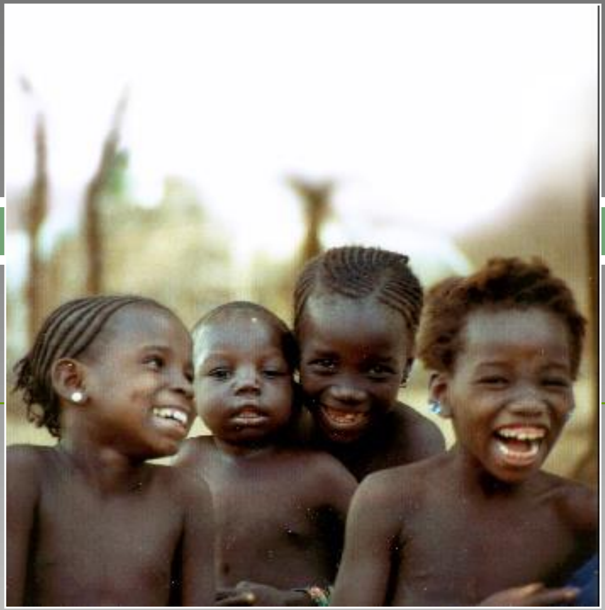
Position on array = fungal species



Single DNA isolation — ~2 h

PRC reactions — ~ 2 h

Fungal species identifications



Thanks for your attention!

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http://charter.mycokey.eu/

ISM – MYCOKEY Workshop - Training Course

"Strategies for minimization of mycotoxins and toxigenic fungi in food chains"

Thanks for your kind attention !!