

# **Risk assessment of fungal biocontrol agents – How can RAFBCA help?**

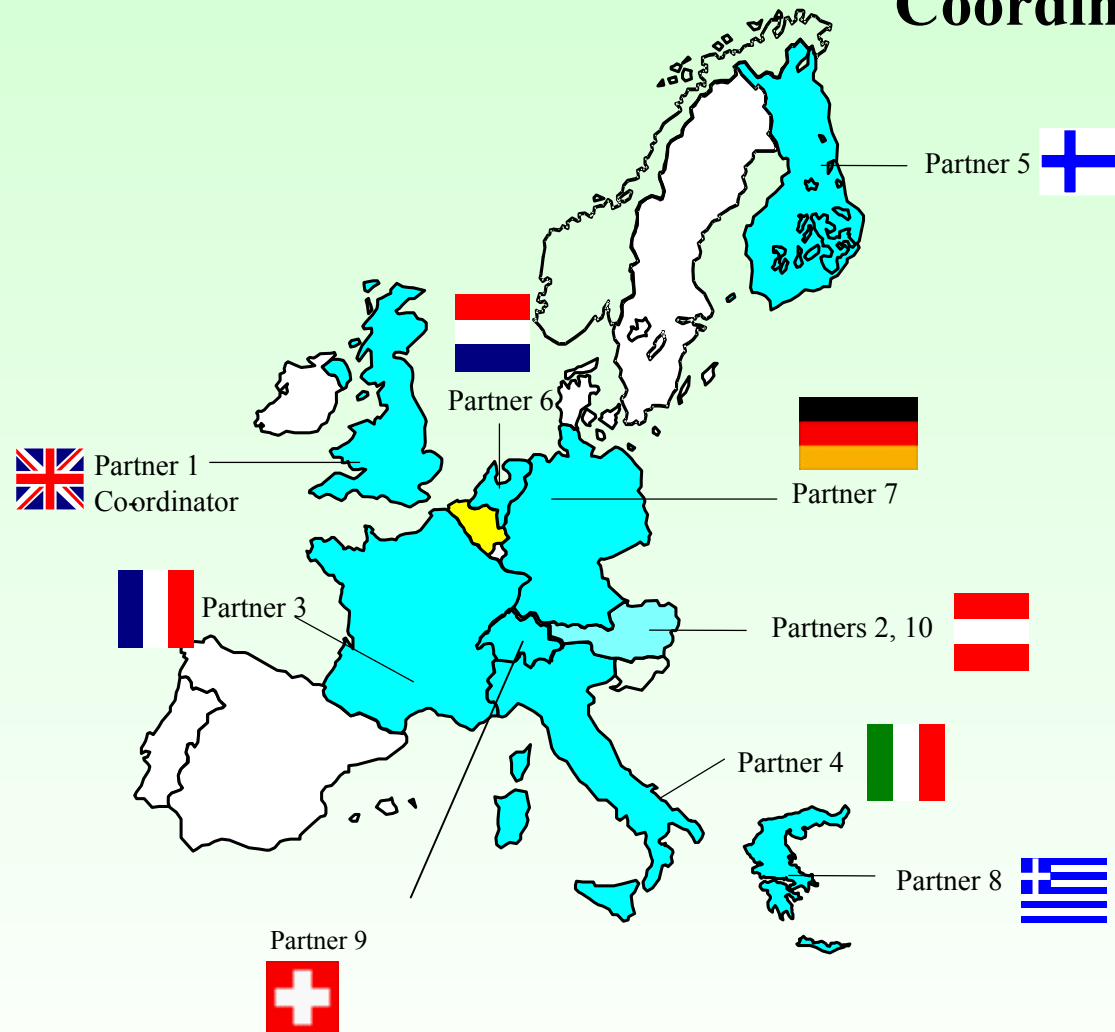
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**Coordinator: Dr. Tariq M. Butt**



## **SMEs**

**Prophyta, Germany**

**Koppert, Netherlands**

**Kemira, Finland**

**Kwizda, Austria**

# RAFBCA Objectives = Workpackages

## WP

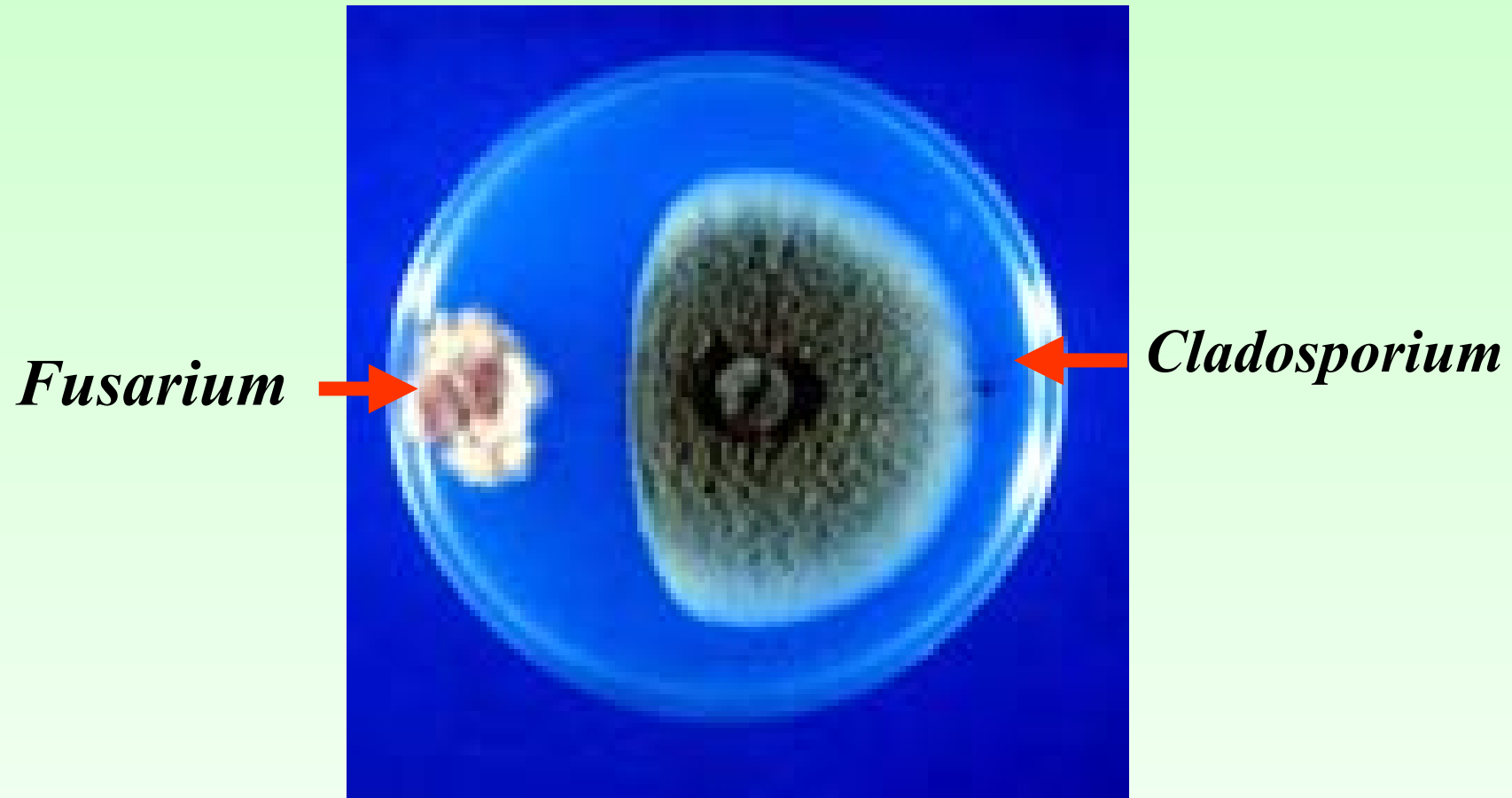
- 1. Identify the major metabolites secreted by fungal BCAs (- i.e. those most likely to enter the food chain)**
- 2. Develop the methods and tools to monitor metabolites and fungal BCAs in the environment**
- 3. Develop *in vitro* toxicity assays such as sensitive cell lines (i.e. biosensors) to detect selected metabolites**
- 4. Determine the role and mode of action of metabolites to identify target sites and potential risks**
- 5. Monitor major metabolites in the environment to see if they enter the food chain. Evaluate the risks they pose to human and animal health if any.**

# **WP1 Identify the major metabolites secreted by fungal BCAs - i.e. those most likely to enter the food chain**

- **Purify & characterise major metabolites secreted by commercially viable strains of BCA**
- **Determine their role**
- **Study regulation of metabolites**
- **Estimate quantities produced *in vivo* and *in vitro***

# Role of fungal metabolites

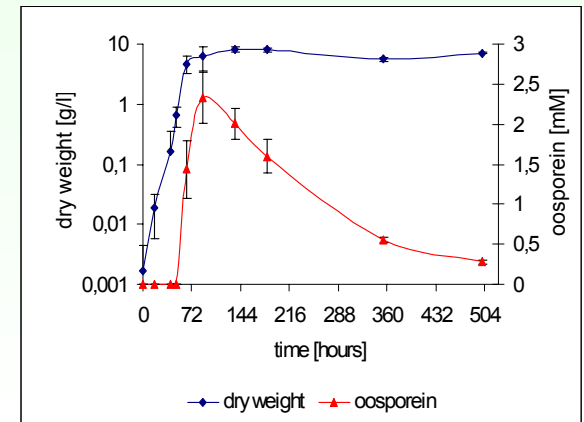
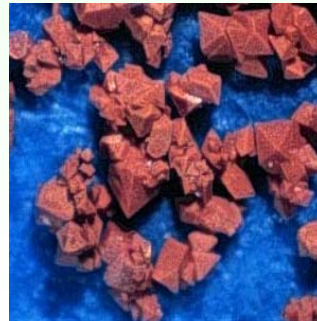
- **Antagonism/Competition** - To displace/inhibit competitor. This enables the BCA to compete for nutrients and space.
- **Survival** - To protect BCA against antagonistic microorganisms or mycophagous organisms



**Antifungal metabolites of *Fusarium* diffuse into medium and inhibit *Cladosporium fulvum***

# Intra-specific variation in Oosporein (red-coloured dibenzoquinone) production by *Beauveria brongniartii*

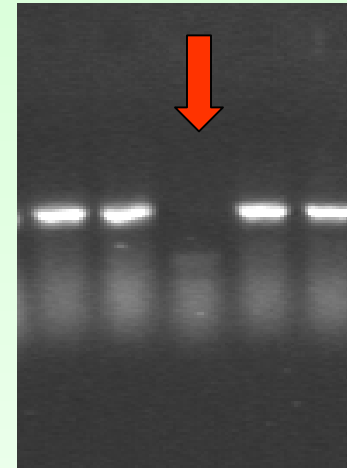
Strain	Oosporein(mg/l)
<i>B. brongniartii</i> – IMBST 95031	28
<i>B. brongniartii</i> – IMBST 95041	270
<i>B. brongniartii</i> – CH 95011	95
<i>B. brongniartii</i> – HLX522	26



## WP2 Develop methodologies and tools to monitor metabolites and fungal BCAs in the environment

- **Species-specific probes**

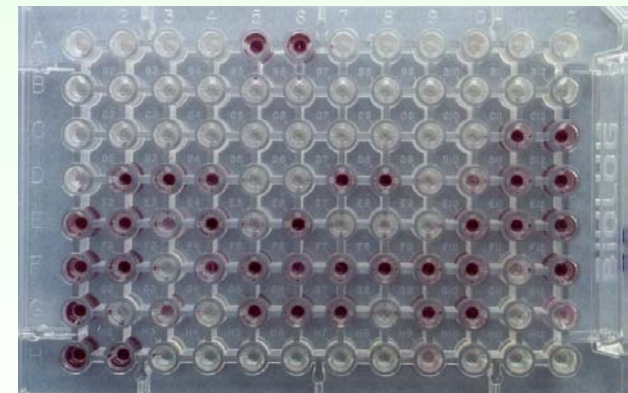
- *Metarhizium*-specific primers based on conserved regions for the species, within the IGS region of rDNA, which were variable for a range of other soil filamentous fungi.



All *M. anisopliae* strains yield a single 380bp PCR product

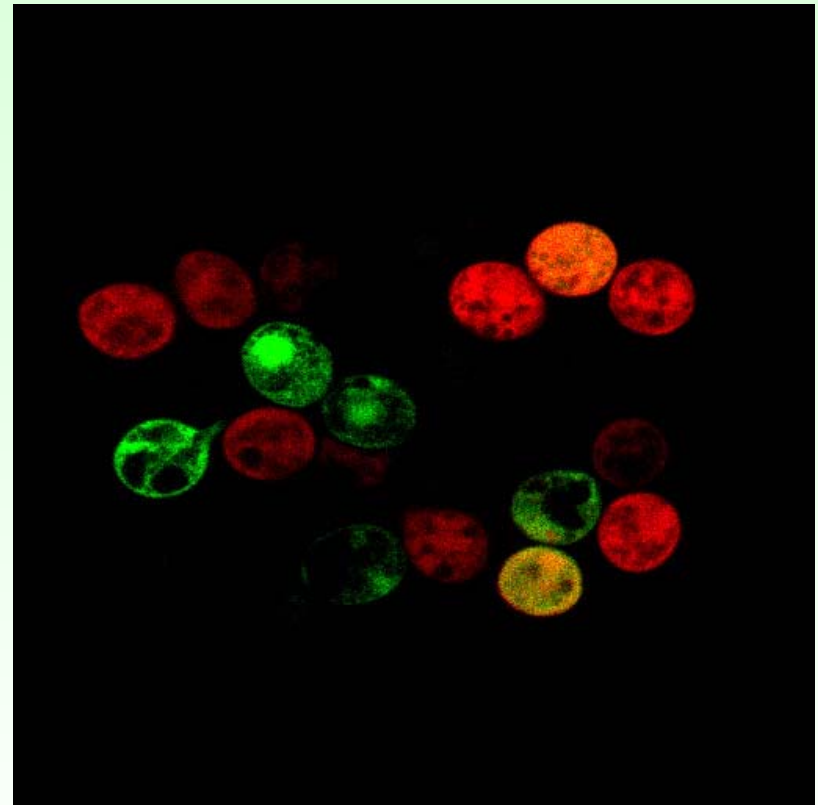
- **Develop high throughput assays**

- detect/quantify metabolites
- determine cytotoxicity and genotoxicity



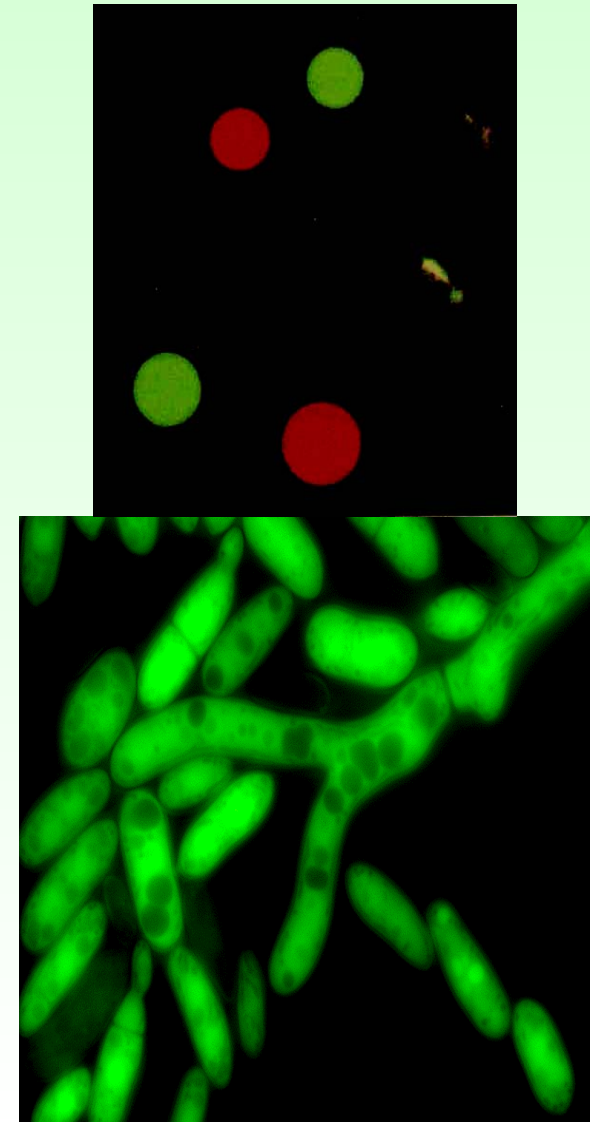
## **WP3 Identify sensitive cell lines (i.e. biosensors) to detect selected metabolites**

- **Identify most sensitive animal, plant and microbial cell lines which can be exploited as biosensors**
- **Determine LD<sub>50</sub> of each compound against most sensitive organism/cell line**



# **WP4 Determine mode of action of metabolites to identify target sites and potential risks**

- **Identify target sites in cells**
- **Adapt probes (e.g. fluorescence probes) for high throughput microtitre assays to screen bioactive compounds with specific activities (e.g. cell lysis, mitotic inhibitors)**



## **WP5 Monitor major metabolites in the environment to see if they enter the food chain**

- 1. Identify and quantify metabolites – crop, soil, target host**
- 2. Determine stability and fate of metabolites in the environment**
- 3. Prepare recommendations on risks of metabolites of beneficial fungal BCAs**

**Maximum amount of oosporein produced by  
*Beauveria brongniartii* - *in situ*, *in vivo* and *in vitro***

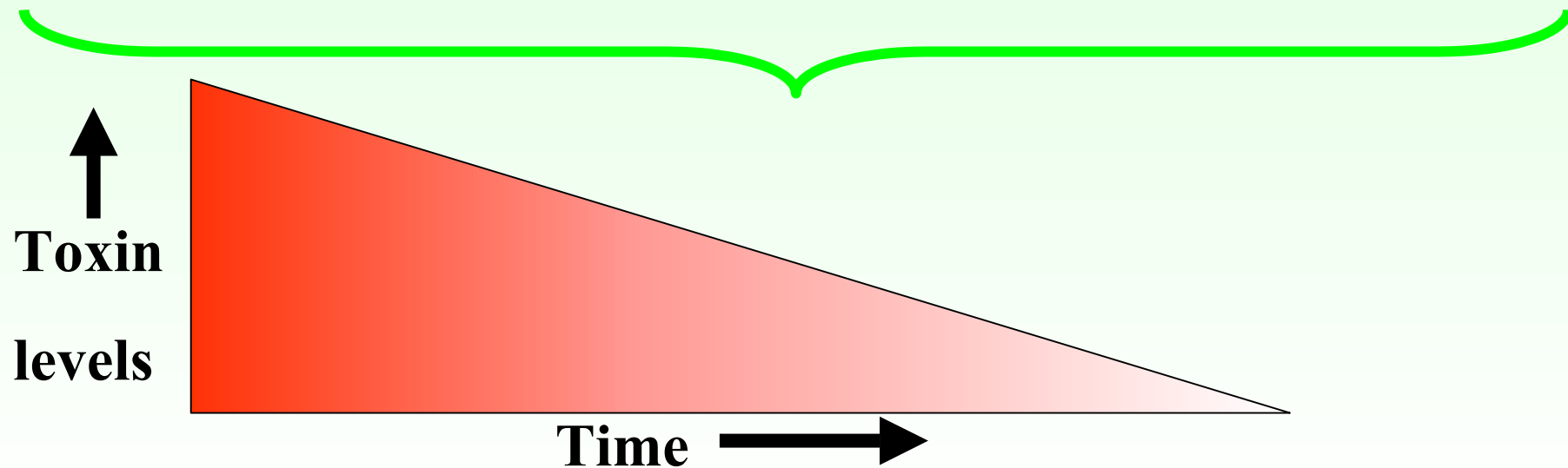
<b>Substrate</b>	<b>Oosporein</b>
<b>Culture media</b>	<b>300 mg/l</b>
<b>Commercial product (Melocont<sup>®</sup>)</b>	<b>3.2 mg/kg</b>
<b>Mycosed chafer larvae</b>	<b>200 µg/larva</b>
<b>Plant biomass</b>	<b>ND</b>
<b>Soil (enrichment via BCA)</b>	<b>0.02 mg/m<sup>2</sup></b>
<b>Soil (enrichment via larvae)</b>	<b>6.4 mg/m<sup>2</sup></b>

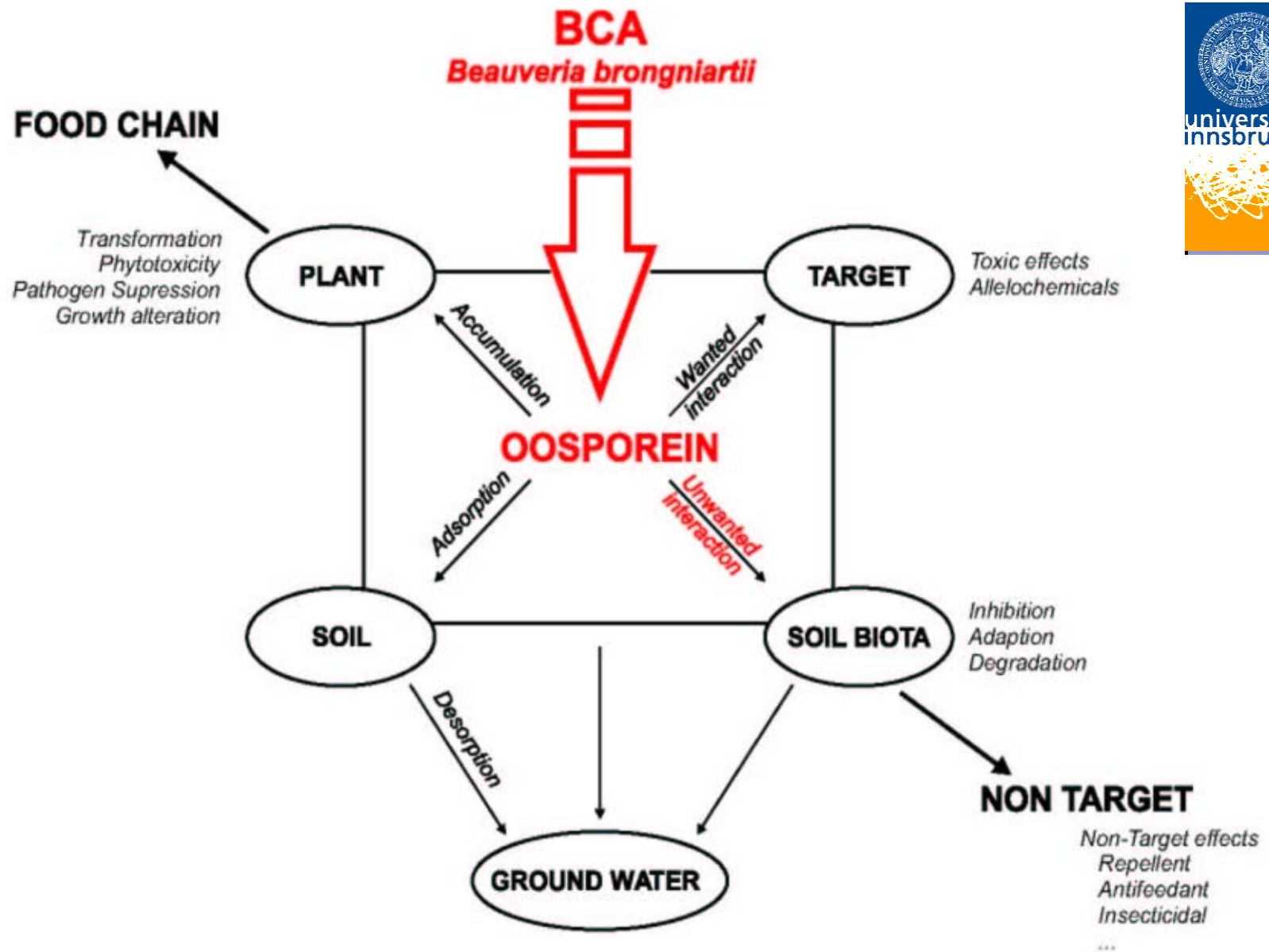
# Environmental factors will influence the persistence of fungal metabolites (toxins)

**Biotic** - metabolites destroyed or exploited by plant/soil microbes

**Edaphic** - metabolites adsorbed onto soil particles/inactivated due to soil chemistry (e.g. pH)

**Climatic** - metabolites inactivated by UV or heat, diluted by rain





# Summary

- **RAFBCA will develop the methods and tools to determine whether fungal BCA metabolites enter the food chain and pose a risk to human and animal health**
- **Expand our knowledge base of fungal metabolites -**
  - *Elucidation of the mode of action of key metabolites*
  - *Data on cytotoxic & carcinogenic properties of key metabolites*
  - *Information on metabolite production*
- **Prepare recommendations for improving registration procedures that will benefit registration authorities and industry**
- **Reduces costs of risk assessment trials - helps make SMEs more competitive**