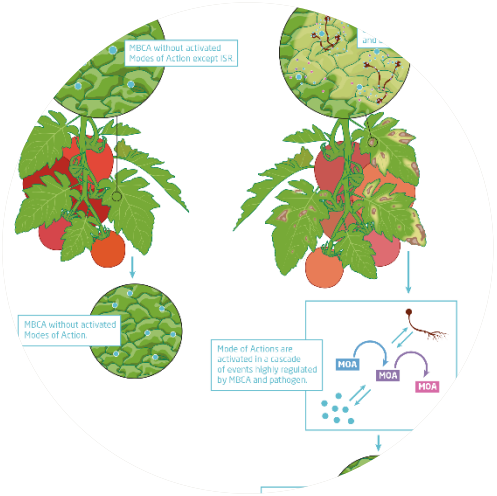
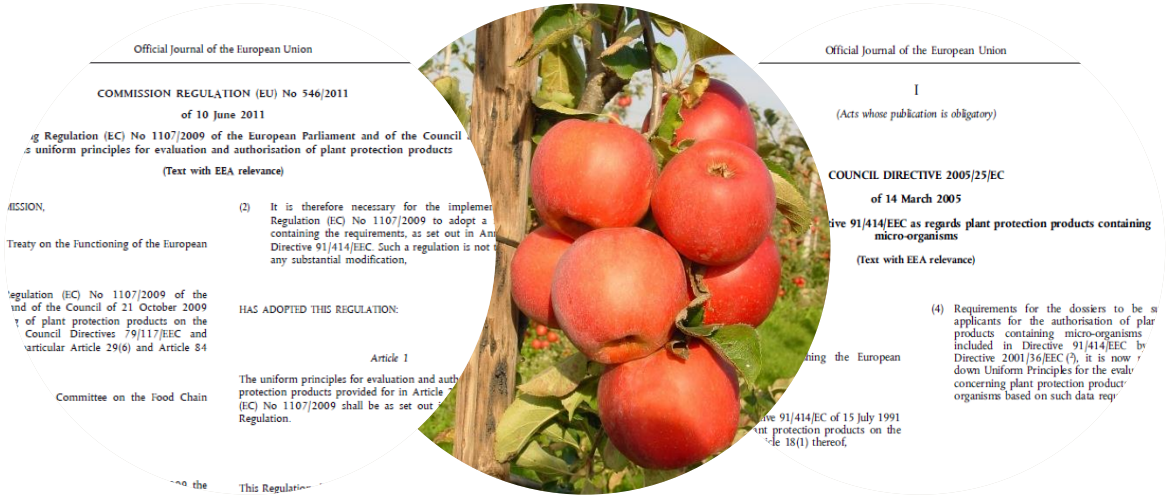
The microbial modes of action against plant pathogens

ABIM 2019, 21 – 23 October 2019, Basel, Switzerland

Jürgen Köhl, Rogier Kolnaar[1] & Willem Ravensberg[2]

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COMMISSION REGULATION (EU) No 546/2011

of 10 June 2011

Regulation (EC) No 1107/2009 of the European Parliament and of the Council
uniform principles for evaluation and authorisation of plant protection products

(Text with EEA relevance)

Treaty on the Functioning of the European

Regulation (EC) No 1107/2009 of the
and of the Council of 21 October 2009
of plant protection products on the
Council Directives 79/117/EEC and
particular Article 29(6) and Article 84

Committee on the Food Chain

(2) It is therefore necessary for the implemen
Regulation (EC) No 1107/2009 to adopt a
containing the requirements, as set out in An
Directive 91/414/EEC. Such a regulation is not
any substantial modification,

HAS ADOPTED THIS REGULATION:

Article 1

The uniform principles for evaluation and auth
protection products provided for in Article
(EC) No 1107/2009 shall be as set out
Regulation.

This Regulation

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I

(Acts whose publication is obligatory)

COUNCIL DIRECTIVE 2005/25/EC

of 14 March 2005

tive 91/414/EEC as regards plant protection products containing micro-organisms

(Text with EEA relevance)

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ive 91/414/EC of 15 July 1991
nt protection products on the
cle 18(1) thereof,

(4) Requirements for the dossiers to be s
applicants for the authorisation of plan
products containing micro-organisms
included in Directive 91/414/EEC by
Directive 2001/36/EEC (3), it is now
down Uniform Principles for the evalu
concerning plant protection product
organisms based on such data requ

[1] Linge Agroconsultancy B.V. [2] Koppert Biological Systems

Why is information on mode of action needed?

Efficacy: **Understanding how BCA works needed to optimize the use**

- Applications strategies
- Timing of application
- Formulation of BCA
- Production of BCA

Why is information on mode of action needed?

EU Regulations

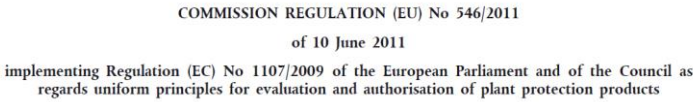
- MOA shall be indicated for the risk assessment
- MOA of produced toxins with effect on target organisms
- Aspects to be considered: antibiosis, induction of plant resistance, interference with virulence of pathogens, endophytic growth, root colonization, competition, parasitization
- Ideally cell factory directly at spot where target organisms are harmful


REGULATIONS

COMMISSION REGULATION (EU) No 283/2013

of 1 March 2013

setting out the data requirements for active substances, in accordance with Regulation (EC) 1107/2009 of the European Parliament and of the Council concerning the placing of pl protection products on the market


COMMISSION REGULATION (EU) No 546/2011

of 10 June 2011

implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products

The modes of action of BCAs against pathogens

Interaction via plant metabolism

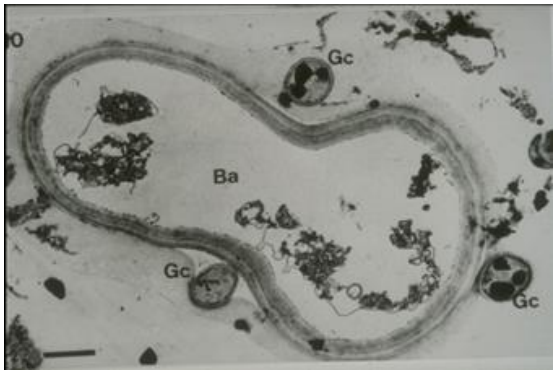
- Induced resistance and priming

Indirect interaction with pathogens

- Competition for nutrients
- Competition for space
- Change of pH

Direct interaction with pathogen

- Hyperparasitism
- Antibiosis by antimicrobial metabolites



Scientific information on mode of action (1)

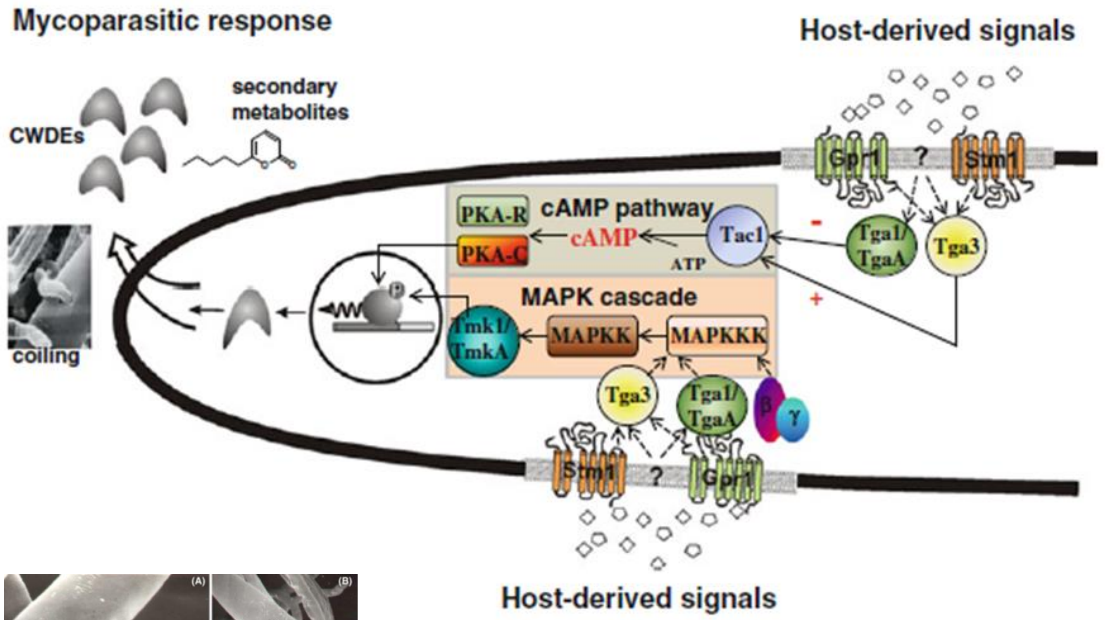
Major reviews

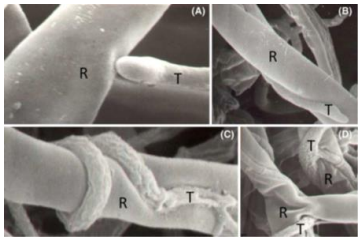
- Compant et al., 2005 — PGPB in biocontrol
- Lugtenberg and Kamilova, 2009 — Rhizobacteria
- Raaijmakers and Mazzola, 2012 — Antibiotics
- Pieterse et al., 2014 — Induced resistance
- Conrath et al., 2015 — Priming
- Massart et al., 2015 — Omic technologies
- Spadaro and Droby, 2016 — Yeasts
- Karlsson et al., 2017 — Mycoparasites
- Mauch-Mani et al., 2017 — Priming
- Ghorbanpour et al., 2018 — Beneficial fungi

Scientific information on mode of action (2)

Major conclusions

- Detailed knowledge
- *In situ*
- Complex
- Regulated
- Cascades of events
- Sequences of different modes of action





Mukherjee et al. 2012. Trichoderma–Plant–Pathogen Interactions: Advances in Genetics of Biological Control. doi: 10.1007/s12088-012-0308-5

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Scientific information (3)

Major conclusions

- Detailed knowledge
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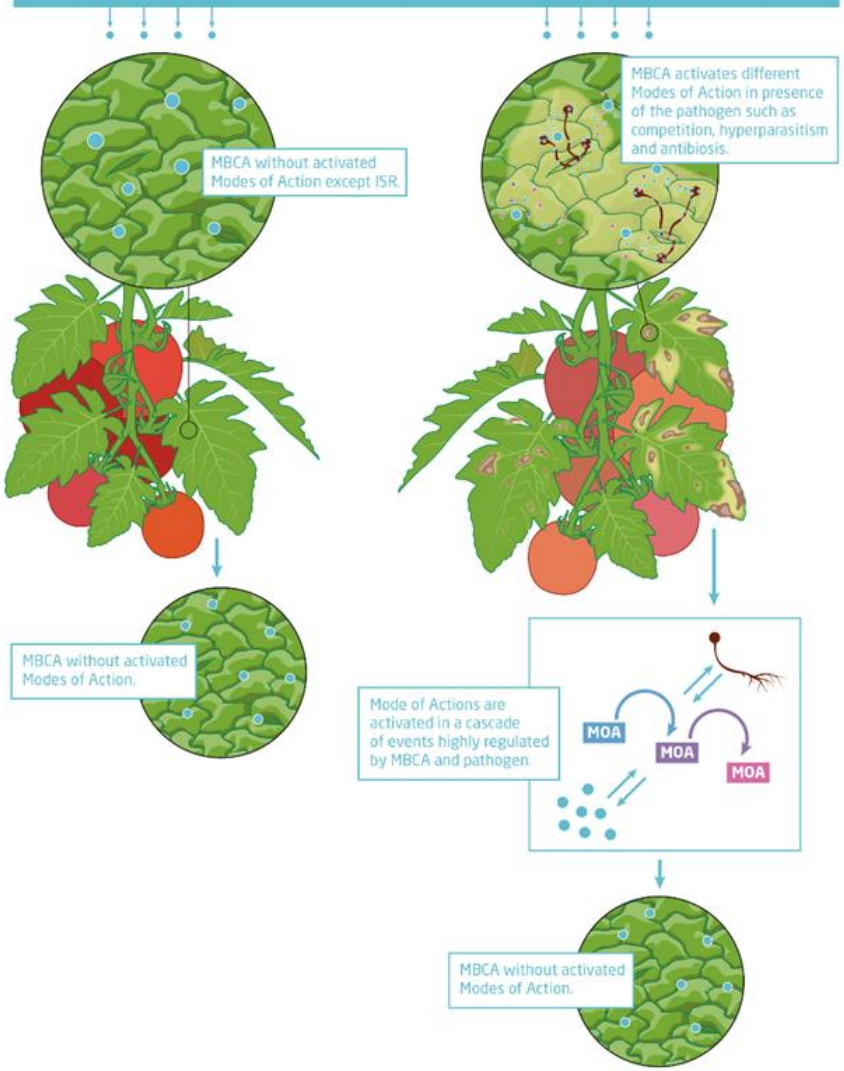

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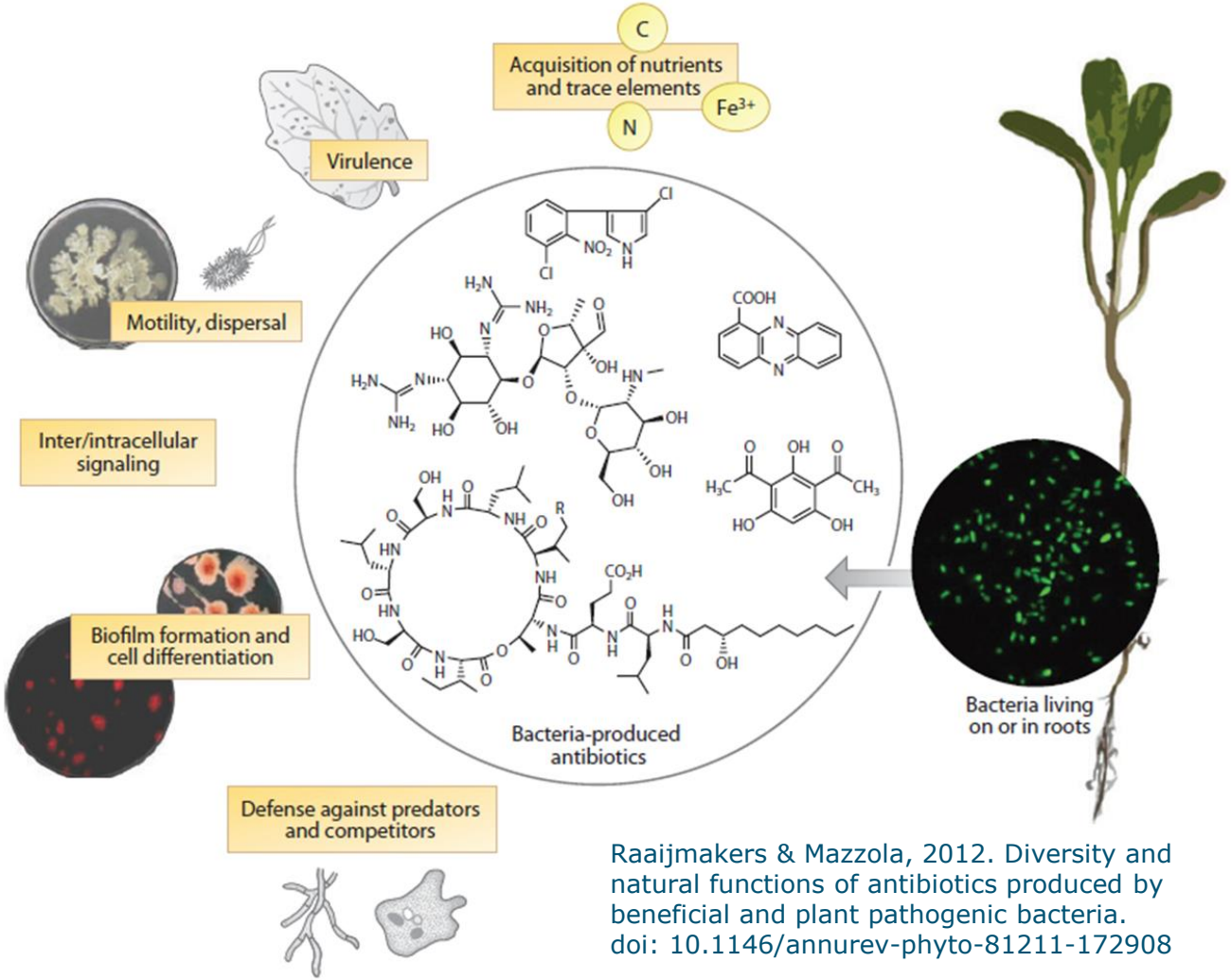
Antibiotic compounds in biological control (1)

- *In situ* in micro niches
- Various functions
- Low concentrations
- Short lifespan
- Huge variety produced in natural environment



Raaijmakers & Mazzola, 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. doi: 10.1146/annurev-phyto-81211-172908

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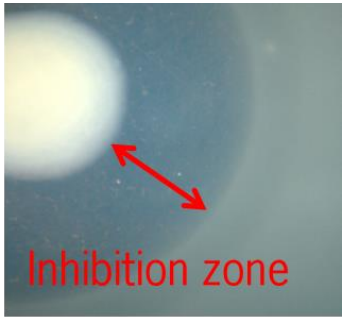
Antibiotic compounds in biological control (2)

➔ *In situ:* temporarily produced at very low amounts with various functions

- *In vitro*: produced in rich media at high amounts with inhibitory effects under suitable conditions
- Scientists often simplify biocontrol by measuring inhibition zones
- Communications on biocontrol often visualize antagonism by showing inhibition zones

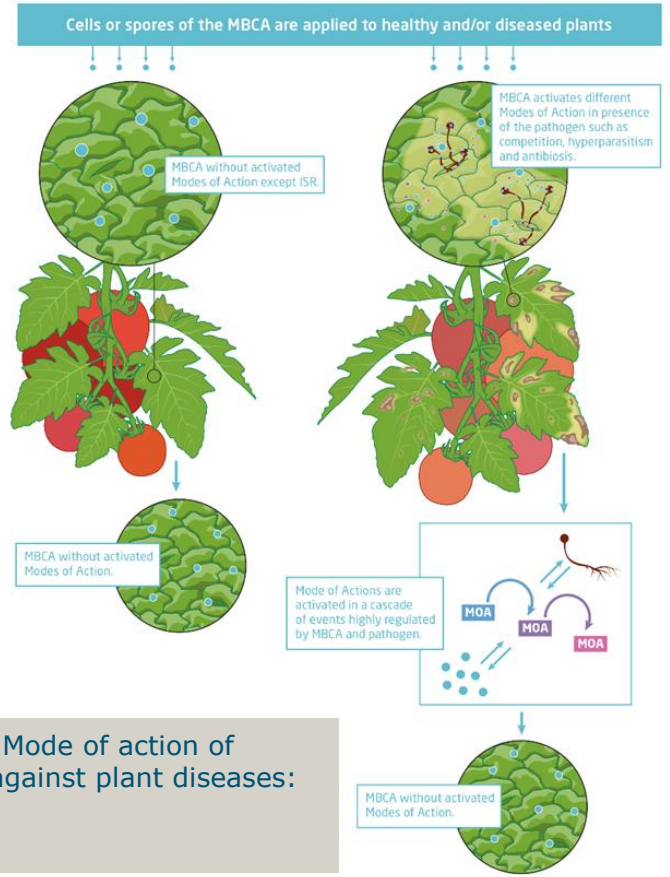
➔ Wrong perception that biological control is based on high amounts of effective antibiotics ?

➔ Wrong emphasis in regulations on role of antibiotics as toxins ?



Mode of action: Relevance beyond efficacy

- Method of screening
- Risk of resistance
- Dependency on environmental conditions
- Dependency on plant physiology
- Potential risks
    - Acute toxicity
    - Metabolites
    - Environmental risks
    - Phytotoxicity



Köhl, Kolnaar & Ravensberg, 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy.
doi: 10.3389/fpls.2019.00845

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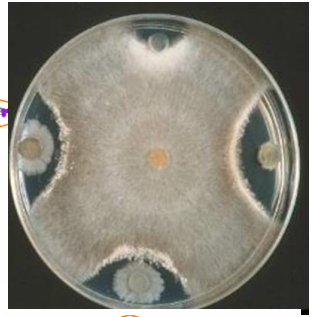
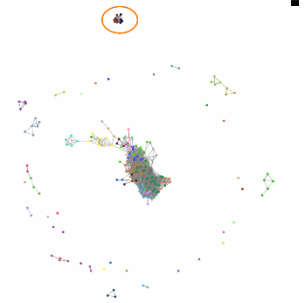
Mode of action	→	Method of screening
• Induced resistance • Competition • Hyperparasitism • Antimicrobial metabolites *in situ*	→	Complex bioassay on plants Simplified bioassays
Antimicrobial metabolite in product	→	*In vitro* assays
Assembled consortia	→	*In silico* design followed by complex bioassays
• Helper strains • Modulation of indigenous microbiota	→	*In silico* design followed by complex bioassays





Mode of action	Risk of resistance	Dependency on environmental conditions	Dependency on plant physiology
• Induced resistance • Competition • Hyperparasitism • Antimicrobial metabolites *in situ*	Low	Low	High
		High	Low
Antimicrobial metabolite in product	Moderate	Low	Low
Assembled consortia	Low	Low	Low
• Helper strains • Modulation of indigenous microbiota	Low	Medium	Low

Mode of action	→	**Risks** Acute toxicity, metabolites, environment, phytotoxicity	→	**Regulations** for authorization of PPPs
• Induced resistance • Competition • Hyperparasitism • Antimicrobial metabolites *in situ*	→	Very low	→	Simplification because of low intrinsic risks
Antimicrobial metabolite in product	→	Risk assessment relevant	→	Use current regulations for PPPs
Assembled consortia	→	Low	→	New concept needed for overall risk assessments
• Helper strains • Modulation of indigenous microbiota	→	Very low	→	No registration required

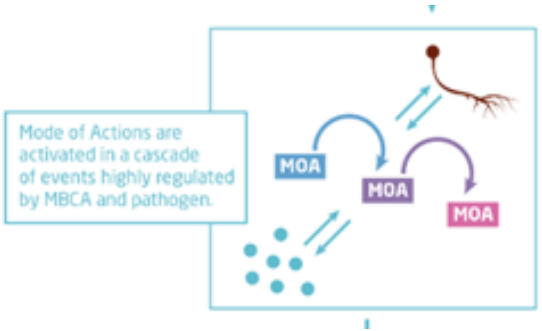
Summary

- MBCAs act via an interplay of different modes of actions but not via a single mode of action

  Multi-omics studies unravel complex events during microbial interactions in the environment

➔ Screening assays needed considering this complex interplay between pathogen and antagonist

- Current EU regulations should regard *in situ* modes of actions as generally safe and not relevant for detailed risk assessments



Thank you for your attention !

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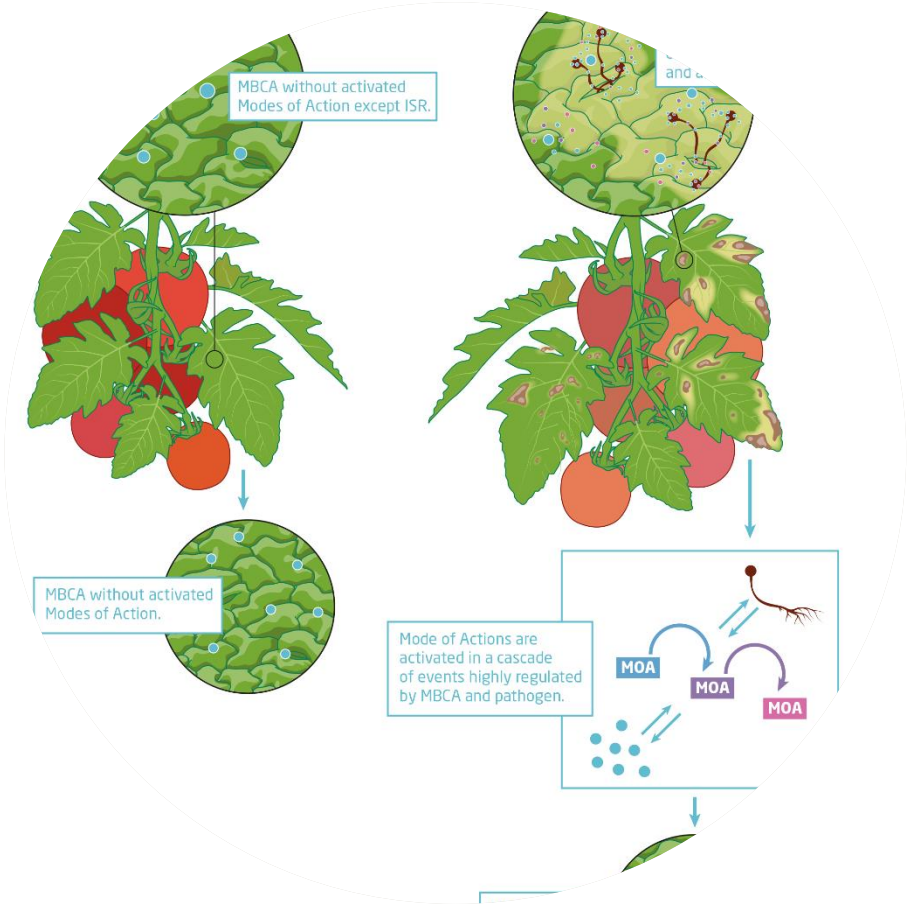

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