Annual Eastern Europe Regulatory Conference

Plant Protection Products – Today and in the Future

29. September – 30. September 2020

Virtual event

INTERNATIONAL YEAR OF PLANT HEALTH 2020

# Room 2 – Session 3
# Introduction to bioprotectants

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FACULTY OF AGRICULTURE AND LIFE SCIENCES UNIVERSITY OF MARIBOR, SLOVENIA

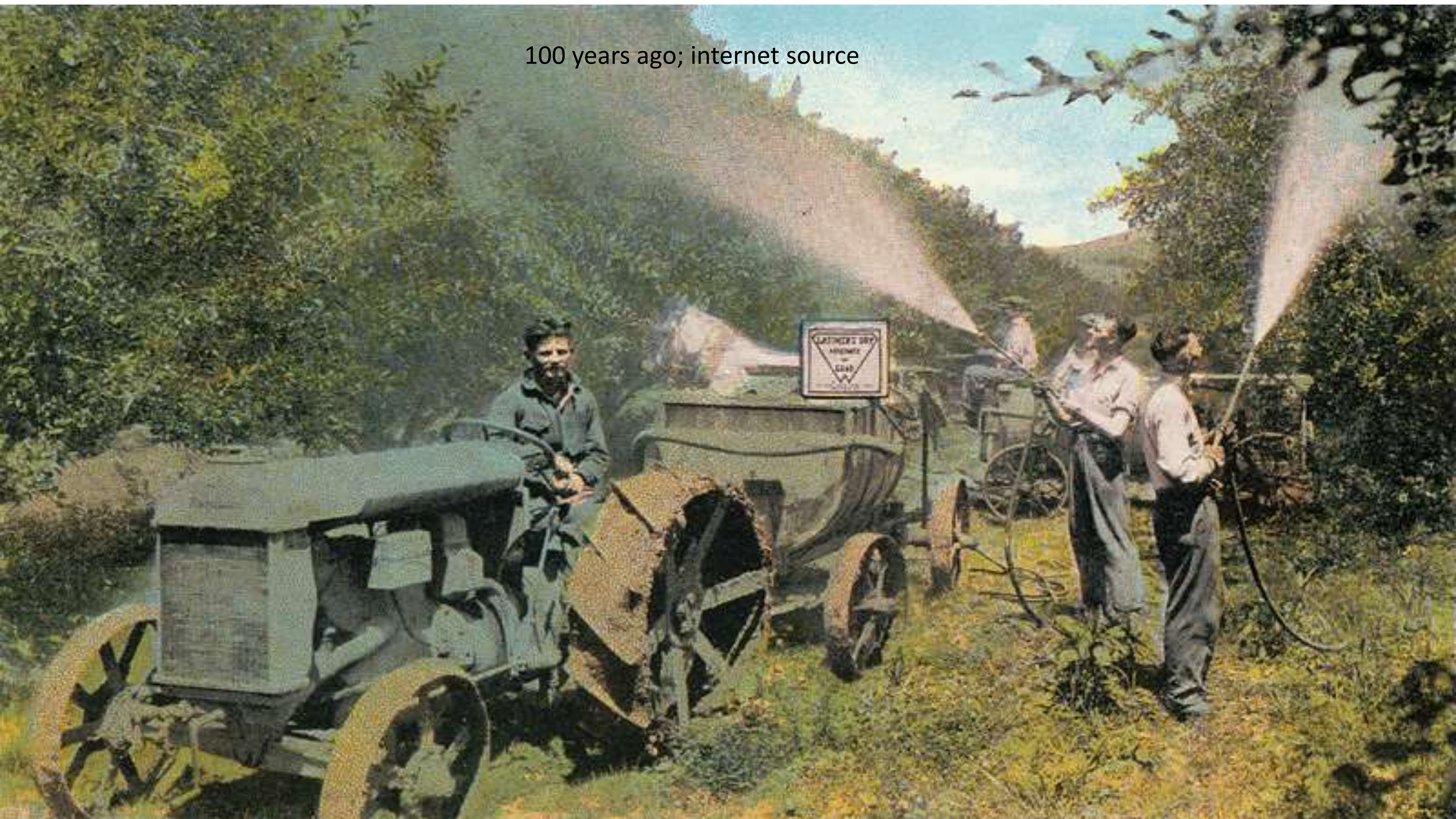
## Coexistence of conventional and bio-products in agriculture

Univerza v Mariboru
Fakulteta za kmetijstvo in biosistemske vede

Our attitude towards pesticides has changed greatly over time,
from a historically uncritical attitude,
to total toxicological paranoia

1800 → 2020

100 years ago; internet source



100 years ago; internet source

# EU DEVELOPMENTS AND NEW EU GREEN DEAL FRAMEWORK

- STRONG WISH TO REPLACE PESTICIDES WITH ALTERNATIVE PRODUCTS
- STRONG WISH TO CHANGE THE RATIO BETWEEN HIGHLY TOXIC AND LOW TOXIC PESTICIDES (PPPs)

## IPM MODIFICATION

FROM:

- MONITOR – DETECT HARMFUL ORGANISM – CHECK ECONOMIC THRESHOLD - CONTROL IT WITH A SPECIFIC PPPs - COMBINE AS MANY AS POSSIBLE DIFFERENT CONTROL TOOLS (focus on how to kill harmful organism safely)

TO:

- CHANGE PLANT CULTIVATION METHODS – CHANGE CULTIVARS – USE BIOSTIMULANTS + BIOLOGICAL CONTROL – MAKE PLANT ABLE TO PROTECT ITSELF - BALANCE EQUILIBRIUM BETWEEN HARMFUL AND BENEFICIAL ORGANISMS – INCREASE COMMON ECOSYSTEM BIODIVERSITY (focus on how to change plants and ecosystem)
- INTRODUCTION OF NEW TYPES OF PLANT PROTECTION PRODUCTS – BIOTECHNOLOGY AND MICROBIOMICS

**A total switch from chemical control to biological control is not realistic**

- **Biological control has limited success if we apply it without changing plant cultivation methods, without new cultivars and without reestablishment of ecosystem equilibrium among harmful organisms and beneficial organisms**

Biological control limits:

- Number of preparations or commercially available organisms is relatively small and spectrum of pests controlled is narrow
- We face resistance of pests also to biological preparations (*B. thuringiensis*, *B. subtilis*, viruses for control of lepidopteran pests, ……….)
- **Obstacles in registration procedures**
- High production costs, wrong policy of agriculture subsidies and plant goods import regulations
- Lack of knowledge of farmers how to apply biological agents correctly and efficiently
- Biological agents can act as invasive organisms

ARCHE CONSULTING

# REGULATORY FRAMEWORK REVIEW

## An analysis of regulation of biological plant protection products and other regulated products

24 September 2018

- Relations   conventional chemical pesticides / bio-pesticides / biostimulants  (abstracted from Arche regulatory framework  review)

- At the moment biological plant protection products (micro-organisms, semiochemicals and botanicals) are still under the scope of PPP regulation 1107/2009.
- Efforts are being made to accommodate these products under the current legislation. There are many proposals to develop **a fast-track registration procedure** on one hand and precaution is advised on other hand as biological PPPs cannot be considered harmless or without risk, just by referring to their natural origin.
- **Pure chemical pesticides are not completely comparable to biological agents. Fate and behaviour in biological systems is quite different.**
- Even bigger gap is between pesticides and biostimulants.
- Gap between biostimulants and bio-pesticides exists too.
- A lot of people dealing with regulatory issues of biological PPPs have the fallowing opinion:
- **Criteria ruling approval of chemical PPPs via the EC 1107/2009 procedure are unsuitable as an authorisation toll for biological PPPs.**
- **Procedures for chemical PPPs are HAZARD orientated, procedures for biological PPPs shell be RISK orientated, if not, marketing is often stopped by to high regulatory burden.**

(abstracted from Arche regulatory framework  review)

We encouter situations on the market where we have products that contain exactly the same organisms or the same botanicals and one are registered as PPPs and other as biostimulants, at the same time.

Depending on the **mode of action**, a biological input can fall under different legislations. For example, if a product works against abiotic stress, it could be considered to fall under the fertilizer legislation as biostimulant once the revised Regulation (EC) 2003/2003 will come into force, but if a product works against biotic stress (such as induced resistance) or against a pest or a disease, it falls under the Plant Protection Products Regulation (EC) No 1107/2009. This is problematic, given the vast difference in data requirements and assessments between both procedures. The more since the **claim** determines which legislation will apply, and not the **intrinsic properties** of the substance and the **exposure profile** of the product.

No EU regulatory framework is currently applicable for biostimulants. Regulation (EU) No 2019/1009, which covers biostimulant approvals, recently entered into force and will be applicable as of 16 July 2022.

Each of these three products could be registered as biological plant protection products or biostimulant



Michał Pylak · Karolina Oszust · Magdalena Frąc

Rev Environ Sci Biotechnol (2019) 18:597–616

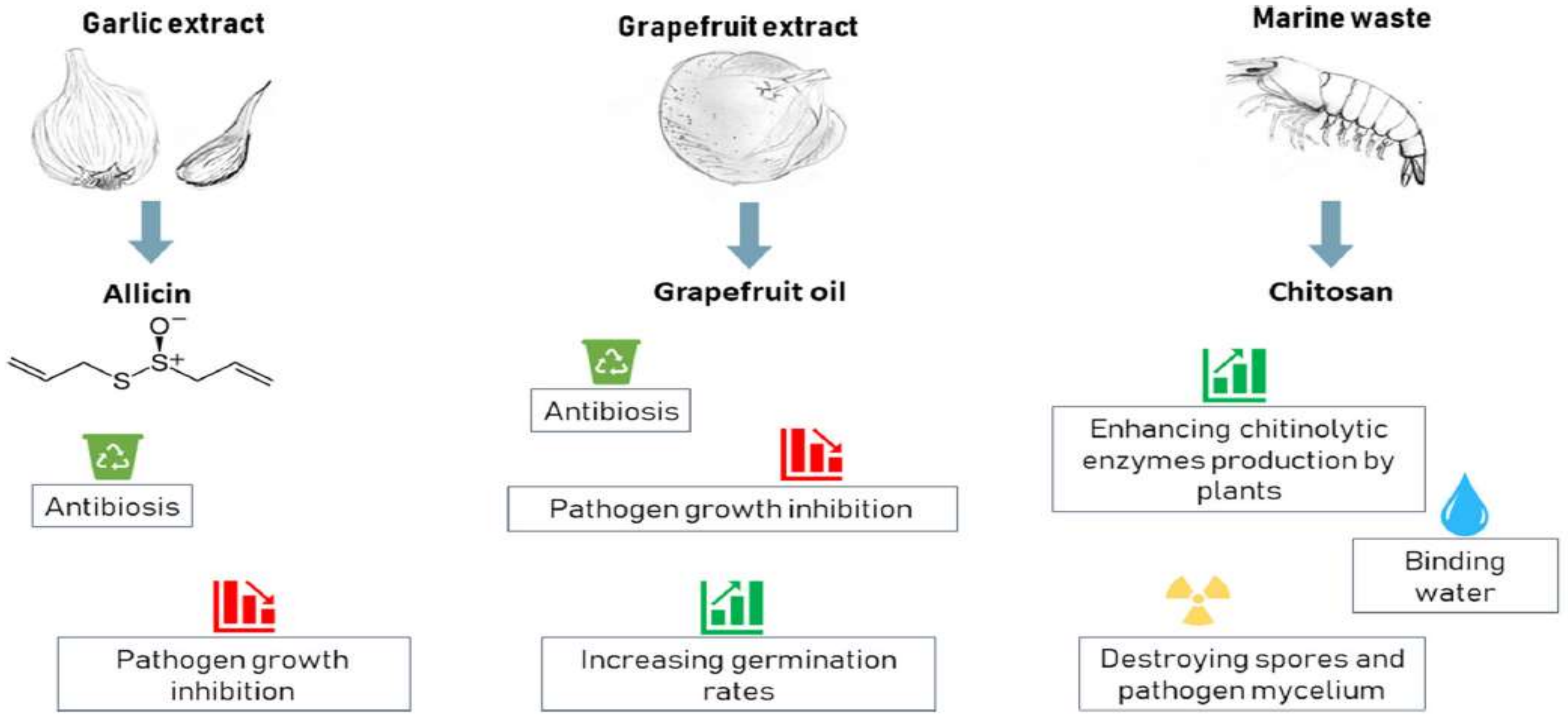



**Fig. 3** Presentation of different sources of bioproducts and properties of such bioproducts

## Can changed plants treated with biological PPPs and biostimulants impact human health ???????

- In nature most of intra- and inter- species fight for survival is based on chemical interactions. Whatever product we use – chemical interactions are behind it and behind its mode of action.
- Modern alternative products change plants, there metabolism and their microbial communities.
- Plant antinutritives impact pathogen and insect reproduction capacity – the same goes for humans - are we aware of that fact????
- - **With modern approach in plant protection  we turn plants back as they were in the beginnig when we started selection of wild plants ??????????**

*Journal of Food Protection Vol. 42, No. 6, Pages 496-501 (June, 1979)*


# Stress Metabolites of Plants — A Growing Concern

**GARNETT E. WOOD**

*Division of Chemistry and Physics, Food and Drug Administration, Washington, D.C. 20204*

(Received for publication August 14, 1978)

**ABSTRACT**

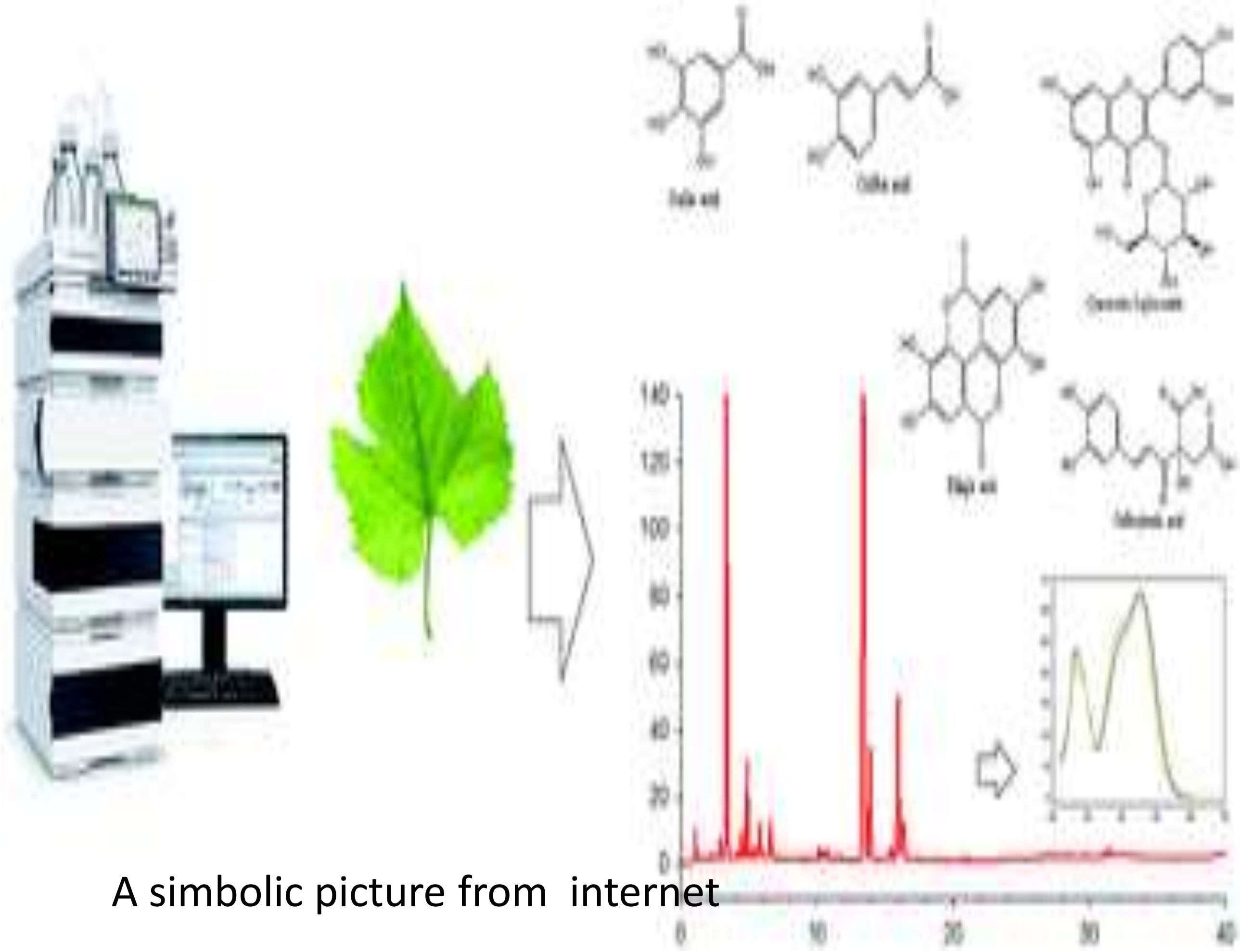
For many years it has been known that plants, when subjected to stress, produce "unusual" metabolites in response to that stress. Some of these metabolites are toxic and, consequently are of concern from the standpoint of contamination of foods. Each year additional stress metabolites are isolated and identified from plants and plant products subjected to stress conditions; these conditions include infection of the plant by microorganisms, mechanical damage during processing or storage, exposure to temperature extremes and the like. The need for an intensive research effort into formation, isolation and characterization, and toxicological evaluation of such metabolites in plants used for human food is discussed.

objectives of this discussion are (*a*) to account for some of the stress compounds produced in other plant families used for food and (*b*) to correlate their presence with concerns for the safety of humans consuming these plants and/or their products as food.

## LEGUMINOSAE

Many species of plants belonging to the Leguminosae family are included in the daily diets of people throughout the world. It is now well established that many legumes are used as additional protein sources for humans. No attempt will be made to mention all of the

Application of bio-pesticides, biostimulants and other alternative agents causes the formation of pesticide metabolites or plant metabolites whose toxicological effects are unknown.



A simbolic picture from  internet

Application of bio-pesticides, biostimulants  and other alternative agents causes the formation of pesticide metabolites or plant metabolites whose toxicological effects are unknown.

Europeans  do not want to consume genetically modified plants

Are we willing to consume plants modified by biostimulator which can start to produce metabolites that didn't exist in specific types of plants before???????
MAYBE  WE NEED TO  INTRODUCE  MRL  FOR  PLANT METABOLITES

A big question remains of how to deal with microbial agent metabolites??

We still do not pay attention to plant metabolites.

We still do not pay attention to plant and human microbiome metabolites.

OECD

Organisation for Economic Co-operation and Development

ENV/JM/MONO(2018)33/ANN1

Unclassified English - Or. English

21 November 2018

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

**Hypotheses for formation secondary metabolites**

Biological control agents produce a wide array of SMs. The question is often posed why these SMs are being produced. There are several reasons:

- Defence: these SMs are a defence against the immune system of the parasitized insect/plant pathogen.
- Antagonism: these SMs have antibiotic properties, including against competing microbials.
- Competition: these SMs enable a mciroorganism to compete for nutrients and space.
- Pathogenesis: other SMs may be important pathogenicity determinants (Amiri et al., 1999; Bandani et al., 2000). They kill or weaken the host.

Annex to the Working Document on the Risk Assessment of Secondary Metabolites of Microbial Biocontrol Agents

Series on Pesticides
No. 98

Reviews of Environmental Contamination and Toxicology pp 47-137 | Cite as

# Natural Pesticides and Bioactive Components in Foods

Authors    Authors and affiliations

Ross C. Beier



Part of the Reviews of Environmental Contamination and Toxicology book series (RECT, volume 113)

## Abstract

The purpose of exploring the potential naturally occurring toxic hazards in food plants is not to persuade a person to avoid these common foods. Rather, it is important to put some perspective on these chemicals (natural pesticides) in our foods, and to clearly show that their toxicology, which is unknown in most cases, needs to be better understood. Many natural pesticides function similarly to synthetic pesticides or other biohazard chemicals. The natural pesticide concentration in our foods may be as much as 10,000 times higher than that of synthetic pesticide residues (Ames 1983). In some cases, these natural chemicals are prime candidates to be monitored by plant producers and plant breeders.

VKM Report 2017: 16

# Microorganisms in biostimulants

**Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety**

Problems of contradictory scientific claims on hazard and risks of biological PPPs and biostimulants

## Conclusions:

### Health risks

We do not need to worry at all

Based upon our literature review, we have found no indication of any specific diseases in plants, animals or humans induced by the discussed microorganisms. A few reported cases of human disease are caused through wound infections or injections in immunocompromised patients. These represent a situation where any microorganism may induce infections and is not specific for the agents discussed in this report. In summary, the risk of any disease caused by the discussed microorganisms is considered negligible.

# Standard questions about safety of microbial based biostimulants

For microbial biostimulants the following additional health requirements to prevent unwanted organisms are included:

a) *Salmonella spp.* shall be absent in a 25 g or 25 ml sample of the CE marked fertilising product.

b) *Escherichia coli* shall be absent in a 1 g or 1 ml sample of the CE marked fertilising product.

c) *Enterococcaceae* must not be present in the CE marked fertilising product by more than 10 CFU/g fresh mass.

d) *Listeria monocytogenes* shall be absent in a 25 g or 25 ml sample of the CE marked fertilising product.

e) *Vibrio* spp. shall be absent in a 25 g or 25 ml sample of the CE marked fertilising product.

f) *Shigella* spp. shall be absent in a 25 g or 25 ml sample of the CE marked fertilising product.

g) *Staphylococcus aureus* shall be absent in a 1 g or 1 ml sample of the CE marked fertilising product.

The Food Safety Authority and the Environment Agency wants VKM to answer the following questions for the organisms listed in the positive list:

**1. Health Effects**

1.1. Can the use of these organisms, as fertilising materials, cause adverse effects on plant, animal or human health?

1.2. With the criteria for biostimulants given in the draft regulation, is there any risk that it may follow other organisms with the products that could possibly lead to adverse effects on plant, animal or human health?

**2. Effects on biodiversity and dispersal**

2.1. Is it likely that the relevant organisms may spread to other non-treated areas?

2.2. Can import and use of the relevant organisms cause adverse impacts on biodiversity?

2.3. Are any of the respective organisms not to be regarded as alien species according to the definition in the Norwegian Nature Diversity Act § 3?

**3. Quality of agricultural land**

3.1. Could the use of these microbial biostimulants lead to that the treated area have reduced ability to act as production soil in agriculture in short or long-term perspective?

Problems of contradictory scientific claims on hazard and risks of biological PPPs and biostimulants

**Modulation of Human Immune Response by Fungal Biocontrol Agents**

**We have to worry**

Cibele Konstantinovas, Tiago A. de Oliveira Mendes, Marcos A. Vannier-Santos, Jane Lima-Santos

Although the vast majority of biological control agents is generally regarded as safe for humans and environment, the increased exposure of agriculture workers, and consumer population to fungal substances may affect the immune system.

Those compounds may be associated with both intense stimulation, resulting in IgE-mediated allergy and immune downmodulation induced by molecules such as cyclosporin A and mycotoxins.

This review discusses the potential effects of biocontrol fungal components on human immune responses, possibly associated to infectious, inflammatory diseases, and defective defenses.

# We have to worry??????


BIOPESTICIDES REGISTRATION ACTION DOCUMENT

(*Pantoea agglomerans* strain C9-1 )
(Chemical PC Code 006470)

Problems of contradictory scientific claims on hazard and risks of biological PPPs and biostimulants

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

Pantoea agglomerans strain E325 (006511) Fact Sheet

Summary

Originally isolated in 1994 by researchers at the U.S. Department of Agriculture, Agriculture Research Collection, this naturally-occurring, non-pathogenic bacterium was first identified as Erwinia herbicola.

Following gas chromatography-fatty acid methyl ester (GC-FAME) and substrate analysis, as well as bacterial taxonomy restructuring, this isolate is now considered a strain of Pantoea agglomerans.

I. Target Pests/ Application Sites & Methods

The end use product Bloomtime®, which contains 7.0% of the active ingredient, is used to control fire blight in apples and pears through air blast spray application. This microbial pesticide is applied at 15 to 20 percent bloom followed by a second application at the first petal fall or full bloom.

II. Human Health Effects

No adverse health effects were observed through submitted data reports and public literature. Based on the data submitted and its low toxicological significance, the active ingredient Pantoea agglomerans strain E325 has a toxicity category IV, the lowest level indicating little to no toxic effects at the highest dose. Certain testing requirements have been waived because of these findings.

III. Environmental and Ecological Effects

Based on the submitted data and waiver rationales, environmental fate data (Tier II/III) was not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing.

IV. Regulatory Information

Registered on September 8, 2006 with a commercial FIFIRA section 3 registration and an exemption from the requirement of a tolerance for the bacterium Pantoea agglomerans strain E325, applied to apples and pears.

V. Additional Contact Information

Ombudsman, Biopesticides and Pollution Prevention Division (751 jcm.asm.org/content/45/6/1989

ASM Journals

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Problems of contradictory scientific claims on hazard and risks of biological PPPs and biostimulants

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

We have to worry??????

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Bacteriology

# *Pantoea agglomerans*, a Plant Pathogen Causing Human Disease

Andrea T. Cruz, Andreea C. Cazacu, Coburn H. Allen

DOI: 10.1128/JCM.00632-07

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## ABSTRACT

We present 53 pediatric cases of *Pantoea agglomerans* infections cultured from normally sterile sites in patients seen at a children's hospital over 6 years. Isolates included 23 from the bloodstream, 14 from abscesses, 10 from joints/bones, 4 from the urinary tract, and 1 each from the peritoneum and the thorax. *P. agglomerans* was most associated with penetrating trauma by vegetative material and catheter-related bacteremia.

*Pantoea agglomerans* (formerly *Enterobacter agglomerans*) is a gram-negative aerobic bacillus in the family *Enterobacteriaceae*. All species of the genus *Pantoea* can be isolated from feculent material, plants, and soil (2), where they can be either pathogens or commensals (12). Within the genus, *P. agglomerans* is the most commonly isolated species in humans, resulting in soft tissue or bone/joint infections following penetrating trauma by vegetation (6, 7, 9, 14, 15). *P. agglomerans* bacteremia has also been described in association with the contamination of intravenous fluid (11), total parenteral nutrition (8), the anesthetic agent propofol (3), and blood products (1). However, spontaneously occurring bacteremia has rarely been reported, wi.com/journals/cripe/2018/4158734/?utm_source=google&utm_medium=cpc&utm_campaign=HDW_MRKT_GBL_SUB_ADWO_PAI_DYNA_JOUR_X&gclid=Cj0KCQiAtOjyBRC0ARIsAIpJyGPV_WVr7v_V4trwpFnmMhPokwyMssbrJFTzdg



Problems of contradictory scientific claims on hazard and risks of biological PPPs and biostimulants





We have to worry??????

Case Report | Open Access
Volume 2018 | Article ID 4158734 | 3 pages | https://doi.org/10.1155/2018/4158734

# *Pantoea agglomerans* Infections in Children: Report of Two Cases

Shraddha Siwakoti,[1] Rinku Sah,[1] Rupa Singh Rajbhandari,[2] and Basudha Khanal[1]
Show more

Academic Editor: Larry A. Rhodes

| Received | Revised | Accepted | Published |
|---|---|---|---|
| 15 Sep 2017 | 16 Dec 2017 | 03 Jan 2018 | 21 Jan 2018 |



## Abstract

*Introduction. Pantoea agglomerans*, primarily an environmental and agricultural organism has been reported as both commensal and pathogen of humans. We present two case reports of *P. agglomerans* infections in children that involved the meninges and b...stream. *Case Presentations.* A 6-month-old female baby, diagnosed as congenital ...cephalus secondary to aqueduct stenosis with ventriculoperitoneal shunt in situ, ...ted 14 days back was brought to the pediatric emergency with a two-day history of ...ever associated with vomiting, irritability, excessive crying, and decreased feeding. Postoperative meningitis was confirmed as cerebrospinal fluid culture revealed *P. agglomerans*. She responded well with a 14-day intravenous (IV) course of ceftriaxone. Also, we report a case of a 3-year-old male child referred to our center with a provisional diagnosis of UTI with chickenpox for further evaluation. During his 24-hour stay at the local hospital, he had received oral antibiotics and urinary catherization. Urine culture of catheter clamp urine was sterile. *P. agglomerans* was grown in blood culture. He was treated successfully with IV ceftriaxone and amikacin. *Conclusion. P. agglomerans* can cause postsurgical meningitis and bloodstream infection in children. The clinical course of infection was mild and timely administration of proper antibiotic resulted in a favorable outcome.

## 1. Introduction

Increased fear on effects of plant hormones on humans

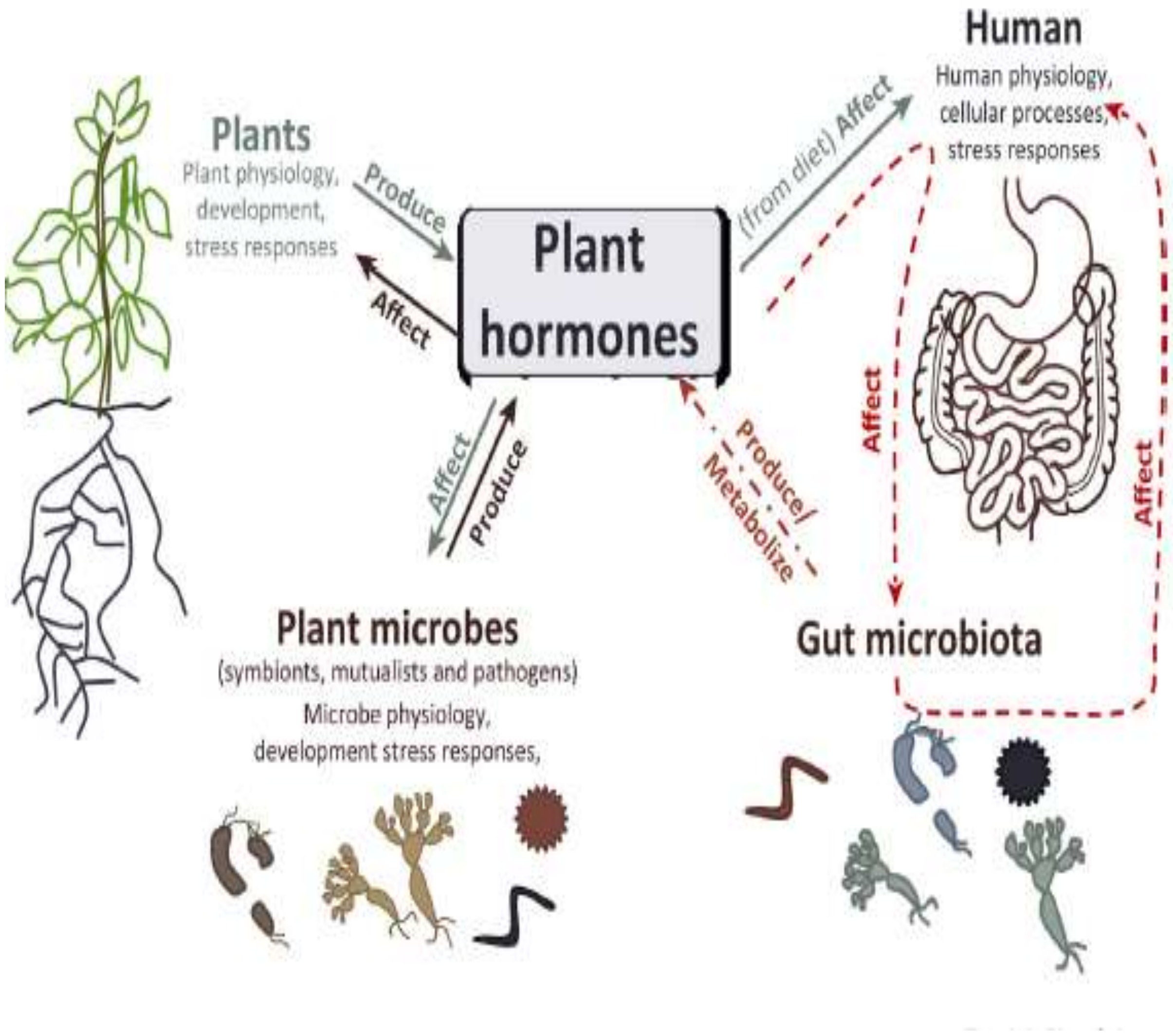
By consuming fruits and vegetables we are eating plant hormones every day

Modern alternative plants protection products contain hormones and stimulate plants to increase hormone production

New classes of plant hormones exist which we do not know

Trends in Plant Science

- Plant Hormones: Key Players in Gut Microbiota and Human Diseases?
- published:August 22, 2017DOI:https://doi.org/10.1016/j.tplants.2017.07.003
- EMILIE CHANCLUD
- **OPINION**| VOLUME 22, ISSUE 9, P754-758, SEPTEMBER 01, 2017




## How humans and their gut microbes may respond to plant hormones

A bowl of salad contains more than vitamins and minerals. Plant matter also includes remnants of the hormones plants produce to control how they grow, age, and manage water intake. Recently, scientists have reported that our gut microbes and cells may respond to these hormones and even produce similar molecules of their own. In an opinion article published August 22 in the journal Trends in Plant Science, researchers in France explore how plant hormones may influence human health.

"We know that gut microbiota are involved in human diseases, and that microbes can biosynthesize plant hormones that affect humans, so it makes sense to investigate animal-microbe interactions from the perspective of plants," says senior author Benoît Lacombe of France's Centre National de la Recherche Scientifique.

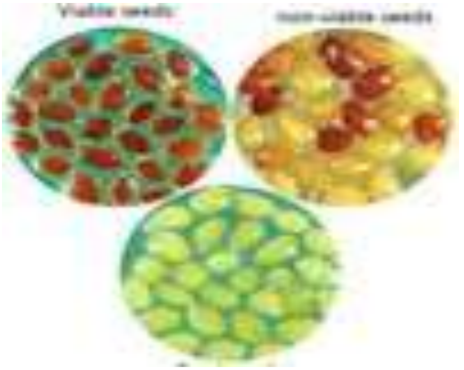
Hormone activity of biostimulants is often clearly visible in fruit

Changes in shape and colour of Golden delicious apples treated with seaweed extracts 6 x a season

Hormone activity of biostimulants is often clearly visible in fruit
Changes in shape and colour of Fuji  apples treated with seaweed extracts 4 x a season and 5 times with epibrasinolid plant hormone  based prepration (preparation Epin extra)

**What effect could plant hormones have on humans?**





1 Answer

Rajiv Angrish, Professor Plant Physiology
Answered Jan 25 2016 · Author has **1.7k** answers and **2.2m** answer views
Originally Answered: what effect could plant hormones have on humans?

•Auxins, cytokinins and  gibbrerllins if taken in through the oral route are metabolised and have no effect on humans.

•Among the 'new' category of plant hormones is **salicylic acid**.  Its topical application eradicates  warts.  Taken orally in right doses it is a pain reliever and lowers body fever.

•Brassinosteroids (BRs), a class of plant-specific steroid hormones, control many of the developmental and physiological processes like their animal counterparts, including regulation of gene expression, cell division and expansion, differentiation, programmed cell death, and homeostasis. Recent studies have indicated that these hormones have antiviral, antifungal, antiproliferative, antibacterial, neuroprotective and immunomodulatory properties in animal system. BRs analogues have been reported to have antiviral activity against herpes simplex virus type 1 (HSV-1), arenaviruses as well as against replication of vesicular stomatitis virus (VSV) in Vero cells. Also, antiherpetic activities both in a human conjunctive cell line (IOBA-NHC) and murine herpetic stromal keratitis (HSK) experimental models have been reported. In human cells, anticancer structure-activity relationship of natural BRs revealed their high cytotoxic activity. Since, BRs and their analogues are reported to inhibit cell growth in cancer cell lines, they may be considered as promising phytohormones for potential anticancer drugs. The use of pollens in folk medicine also indicates scope of steroids of plant pollens in medicines. An attempt has been made in this paper to document the information available on the prospects of BRs in therapeutics.

•***One must interpret these effects only as 'incidental' and these, in my considered opinion, have no evolutionary/biological significance.***

frontiers in Plant Science

REVIEW
published: 29 May 2019
doi: 10.3389/fpls.2019.00655

## ANE IMPROVES PLANT GROWTH BY REGULATING PHYTOHORMONE BIOSYNTHESIS IN PLANTS

Phytohormones are low-molecular-weight compounds produced in very small quantities that regulate several physiological and developmental processes in plants (Wally et al., 2013; Wani et al., 2016). The most common phytohormones include auxins (IAA), cytokinins (CK), abscisic acid (ABA), gibberellic acid (GA), ethylene, jasmonic acid (JA), and salicylic acid (SA) (Wani et al., 2016). One reported growth-promoting effect of ANE was ascribed to the presence of a variety of "phytohormone-like substances" (Stirk and Van Staden, 1997; Khan et al., 2009; Craigie, 2011; Sharma et al., 2014; Battacharyya et al., 2015).

There is a wide variation in auxin content in *A. nodosum* extracts reported in the literature. *A. nodosum* was reported to have a high concentration of indole acetic acid (IAA), approximately 50 mg/g of dry extract (Kingman and Moore, 1982; Khan et al., 2009), whereas Maxicrop®, a different commercial product also prepared from *A. nodosum*, contained 6.63 mg of IAA per gram of dried powder (Sanderson et al., 1987). By using ultra-performance liquid chromatography–electrospray tandem mass spectrometry, Wally et al. (2013) confirmed the presence of 25–35 ng of IAA per dry gram of the extract they tested. This variation in auxin content is likely to be a function of the method of extraction and processing, as well as the geographical location of the raw material harvested including any possible seasonal variation (Stirk and Van Staden, 1996; Wally et al., 2013).

# *Ascophyllum nodosum*-Based Biostimulants: Sustainable Applications in Agriculture for the Stimulation of Plant Growth, Stress Tolerance, and Disease Management

*Pushp Sheel Shukla[1], Emily Grace Mantin[1], Mohd Adil[1], Sruti Bajpai[1], Alan T. Critchley[2] and Balakrishnan Prithiviraj[1]**

[1] Marine Bio-products Research Laboratory, Department of Plant, Food and Environmental Sciences, Dalhousie University, Truro, NS, Canada, [2] Research & Development, Acadian Seaplants Limited, Dartmouth, NS, Canada

Typical algae based products are used very frequently


**FIGURE 1 |** *Ascophyllum nodosum* extract (ANE) improves the growth of several crops by different modes of action.

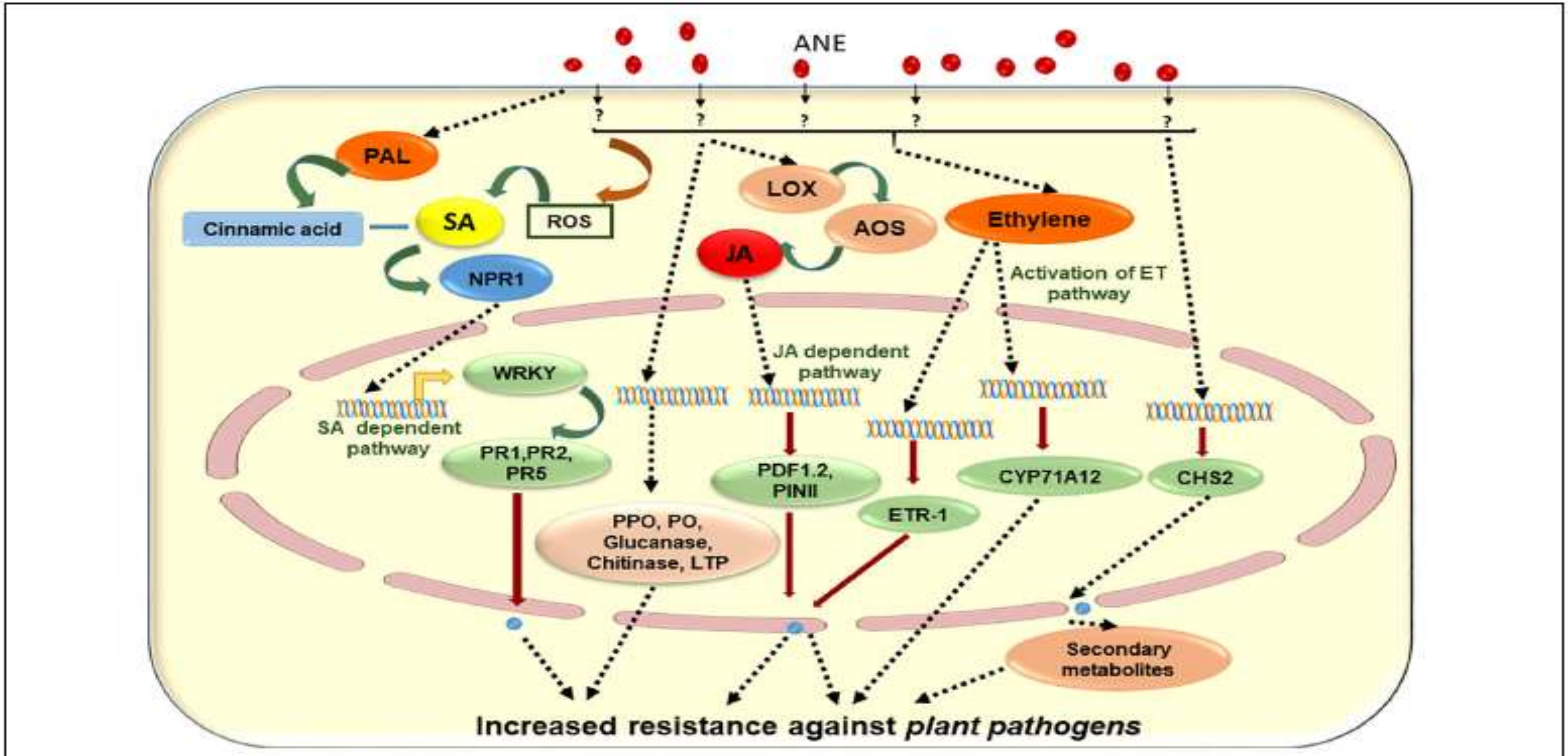



**FIGURE 4 |** Schematic representation of proposed mode of action of *Ascophyllum nodosum* extract (ANE) in eliciting plant defense against different plant pathogens.


# Vidi Parva

*Cold pressed plant extract which consists exclusively of plant-based ingredients like seaweed and herbs*

## Use Vidi Parva for:

Stimulating root growth and improving root quality;

generative plant steering

## Packaging:

Vidi Parva is a liquid formulation sold in 1 litre and 5 litre jerry cans (country specific)

- General information
- A 100% natural plant based biostimulant manufact nodosum). Specifically focused on the stimulation ( horticultural and agricultural crops.
- When to use Vidi Parva?
- Stimulate root and initial plant growth
- Establish and sustain microbial activity, including di
- Increase nutrient uptake
- Increase dry matter content in crop
- Generative steering
- How does Vidi Parva work?
- Vidi Parva stimulates root growth and improves root initiation, therefore providing an id basis for a strong and healthy crop. It also promotes the development of disease-suppressing bacteria aroun roots. Vidi Parva stimulates the formation of root hairs which release exudates to create and maintain th izosphere micro-biology. The product also ensures the development of a compact and resilient plant to support proactive crop balance.

  By applying Vidi Parva, the plant is able to produce auxins more effectively. Auxins are plant hormones that promote root formation. Plants produce auxins with the help of amino acids. Tryptophan, an esse acid in the triggering of natural auxin production is an important component of Vidi Parva. These a xins stimulate the formation of fine roots and root hairs and promote the absorption of nutrients from the rhizosphere. This leads to an increase in the amount of proteins, sugars, and chlorophyll in the crop, resulting in a compact crop with plenty of vitality and vigour.

  In addition to stimulating root initiation, Vidi Parva will proactively assist in the generative steering of the crop. This is a useful tool in maintaining crop balance throughout the season.

6 **Milastin (Biofungicide manufactured by Kan Biosys)**

Just one example of statement from advertisement for biostimulant pointing on hormone action of preparation

Milastin is liquid formulation of dormant forms of multiple plant pro-biotic bacteria Bacillus subtilis. It has multi-level mode of action- Enzymatic, antibiotic secretion, competitive exclusion, plant growth promoting REGULATOR (PGPR) effect and has ZERO Pre-harvest interval (PHI).

Format: Abstract ▾ Send to ▾

Tsitologiia. 2016;58(1):5-15.

# [EFFECTS OF DIFFERENT CLASSES OF PLANT HORMONES ON MAMMALIAN CELLS].

[Article in Russian]

Vildanova MS, Smirnova EA.

## Abstract

Plant hormones are signal molecules of different chemical structure, secreted by plant cells and acting at low concentrations as regulators of plant growth and differentiation. Certain plant hormones are similar to animal hormones or can be produced by animal cells. A number of studies show that the effect of biologically active components of plant origin including plant/phytohormones is much wider than was previously thought, but so far there are no objective criteria for assessing the influence of phytohormones on the physiological state of animal cells. Presented in the survey data show that plant hormones, which have different effects on plant growth and development (jasmonic, abscisic and gibberellic acids), are not neutral to the cells of animal origin, and animal cells response to them may be either positive or negative.

PMID: 27220246

[Indexed for MEDLINE]

- **Phytohormones - hormonally active substances in plants**
- Hormonal substances may occur as natural ingredients in plants. Isoflavones are one example; large levels occur particularly in the soya plant. They are also described as phyto-oestrogens as they can have an impact on the human organism similar to that of the female sexual hormone, oestrogen. They bind to cell receptors like endogenous hormones. This may have different biological consequences in the body. They can contribute to health but can also become a risk. In isolated form isoflavone capsules are available across the counter as a substitute for conventional hormone therapy for the treatment of menopausal disorders in women like hot flushes, night sweats and osteoporosis. Nonetheless, there is no clear scientific evidence that isoflavones do in fact alleviate menopausal disorders; it is far more the case that they may even have undesirable effects. Hence it cannot be ruled out that they may increase the risk of breast cancer as they have an oestrogenic impact on women's breast tissue during menopause.
- At the present time, one subject of controversial debate is whether the hormonal effect of isoflavones on the human organism is the same when isoflavones are ingested from soya-based food as when they are ingested in isolated form as food supplements. A high dose of isolated isoflavones, particularly when it is ingested over a period of several years, could constitute a risk to health.

PHYTOTHERAPY RESEARCH
*Phytother. Res.* **17**, 845–869 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/ptr.1364

*REVIEW ARTICLE*

# Phytoestrogens: a Review of the Present State of Research

**Andreana L. Ososki[1,2,3] and Edward J. Kennelly[1,2*]**

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**Phytoestrogens are a diverse group of plant-derived compounds that structurally or functionally mimic mammalian estrogens and show potential benefits for human health. The number of articles published on phytoestrogens has risen dramatically in the past couple decades. Further research continues to demonstrate the biological complexity of phytoestrogens, which belong to several different chemical classes and act through diverse mechanisms. This paper discusses the classification of phytoestrogens, methods of identification, their proposed mechanisms of action and botanical sources for phytoestrogens. The effects of phytoestrogens on breast and prostate cancers, cardiovascular disease, menopausal symptoms and osteoporosis will also be examined including research on benefits and risks.** 

*Keywords:* botanicals; coumestans; isoflavones; lignans; phytoestrogens.

# [http://e.hormone.tulane.edu/learning/phytoestrogens.html#health_risks](http://e.hormone.tulane.edu/learning/phytoestrogens.html#health_risks)

- Some scientists believe that plants make phytoestrogens as a defense mechanism to stop or limit predation by plant-eating animals (Ehrlich and Raven 1964; Guillette et al. 1995; Hughes 1988). Instead of protecting themselves with thistles or thorns or tasting bad, these plants use chemicals that affect the predatory animal's fertility.
- Although using estrogen-mimicking compounds for protection may sound far-fetched, it makes sense from an evolutionary stance. Many real-life examples support the theory that plants and animals change together, or co-evolve, over time.
- The explanation goes something like this: to avoid predation, plants produce compounds (phytoestrogens) that limit an herbivores reproduction. Thus, the predator's population decreases and more plants can prosper.
- But remember, because of genetic differences, not all species or individuals of a given species will react to the phytoestrogens in the same way. While some herbivores may show fertility problems, others may acquire resistance - like some insects are resistant to pesticides and some bacteria can survive antibiotics. Likewise, some humans may be more susceptible to the benefits and risks of phytoestrogens than others would be.

EXAMPLE OF HIGHLY ACTIVE PLANT EXTRACTS OF PUERARIA  MIRIFICA

HORMONS OF PLANT ORIGIN THAT CAN
SIGNIFICANTLY ALTER HORMON RELATIONS IN HUMAN BODY



toxins

Article

# *Pueraria mirifica* Exerts Estrogenic Effects in the Mammary Gland and Uterus and Promotes Mammary Carcinogenesis in Donryu Rats

Anna Kakehashi [1,*], Midori Yoshida [2,†], Yoshiyuki Tago [1], Naomi Ishii [1], Takahiro Okuno [1], Min Gi [1] and Hideki Wanibuchi [1]

**Abstract:** *Pueraria mirifica* (PM), a plant whose dried and powdered tuberous roots are now widely used in rejuvenating preparations to promote youthfulness in both men and women, may have major estrogenic influence. In this study, we investigated modifying effects of PM at various doses on mammary and endometrial carcinogenesis in female Donryu rats. Firstly, PM administered to ovariectomized animals at doses of 0.03%, 0.3%, and 3% in a phytoestrogen-low diet for 2 weeks caused significant increase in uterus weight. Secondly, a 4 week PM application to non-operated rats at a dose of 3% after 7,12-dimethylbenz[a]anthracene (DMBA) initiation resulted in significant elevation of cell proliferation in the mammary glands. In a third experiment, postpubertal administration of 0.3% (200 mg/kg body weight (b.w.)/day) PM to 5-week-old non-operated animals for 36 weeks following initiation of mammary and endometrial carcinogenesis with DMBA and *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG), respectively, resulted in significant increase of mammary adenocarcinoma incidence. A significant increase of endometrial atypical hyperplasia multiplicity was also observed. Furthermore, PM at doses of 0.3%, and more pronouncedly, at 1% induced dilatation, hemorrhage and inflammation of the uterine wall. In conclusion, postpubertal long-term PM administration to Donryu rats exerts estrogenic effects in the mammary gland and uterus, and at a dose of 200 mg/kg b.w./day was found to promote mammary carcinogenesis initiated by DMBA.

Can botanicals and seaweed based products contain endocrine disruptors???

SHOULD WE INTRODUCE MRL VALUES FOR PLANT METABOLITES ????





Risk communication
Research
Science News
Press office
The press office - contacts
Press releases
2020
2015
2014
2013
2012
2011
2010
2009
2008

# Endocrine disruptors: Substances with harmful effects on the hormone system

A/2010, 19.04.2010

Background information for journalists

"The world is becoming more female" was the title of an article in the magazine in the Süddeutsche Zeitung and it went on "And that's definitely not good news". It referred to studies which reported that reproduction and development disorders have been observed above all in men and male animals. Certain chemicals that can influence the hormone system are thought to be the cause. We ingest them from food and from the air. So-called endocrine disruptors are suspected of promoting the onset of specific tumours which disrupt the development of the human organism or impair reproductive ability. Substances discussed in the public domain, which are suspected of being endocrine, are bisphenol A and certain plasticisers used to manufacturer plastics. But not only synthetically produced chemicals can influence the hormone system but also specific plant ingredients which are found in our food. For instance, soya contains so-called isoflavones which can bind to the receptor for the female sexual hormone, oestrogen. However, dietary intake of substances of this kind does not automatically constitute a health risk. Endocrine disruptors are not a uniform group of substances. The structure and mechanics of the individual substances may differ greatly. Hence they must be viewed in a differentiated manner from the toxicological angle in order to estimate the health risk.

The hormone system is involved in the steering of almost all bodily functions for instance in energy production and use, regulation of blood pressure and electrolyte metabolism. Reactions to emergency situations (hunger, stress, infection) are steered by hormones as are mood, behaviour, growth, development and reproduction. The hormone system is influenced by numerous internal and external factors. Many natural and synthetic substances can impact the hormone system when they reach the body. Critical doses can on the one hand directly disrupt specific hormone-dependent body functions. Some substances can also influence the hormone system during sensitive development phases like, for instance, the unborn child during pregnancy and, in this way, cause permanent damage to health. Synthetic or natural substances of this kind, which may have harmful effects on the hormone system, are called endocrine disruptors.

*Review Article*

# Safety of Natural Insecticides: Toxic Effects on Experimental Animals

Botanical preparations contain many substances that are classified as highly toxic according to human cell culture toxicological tests

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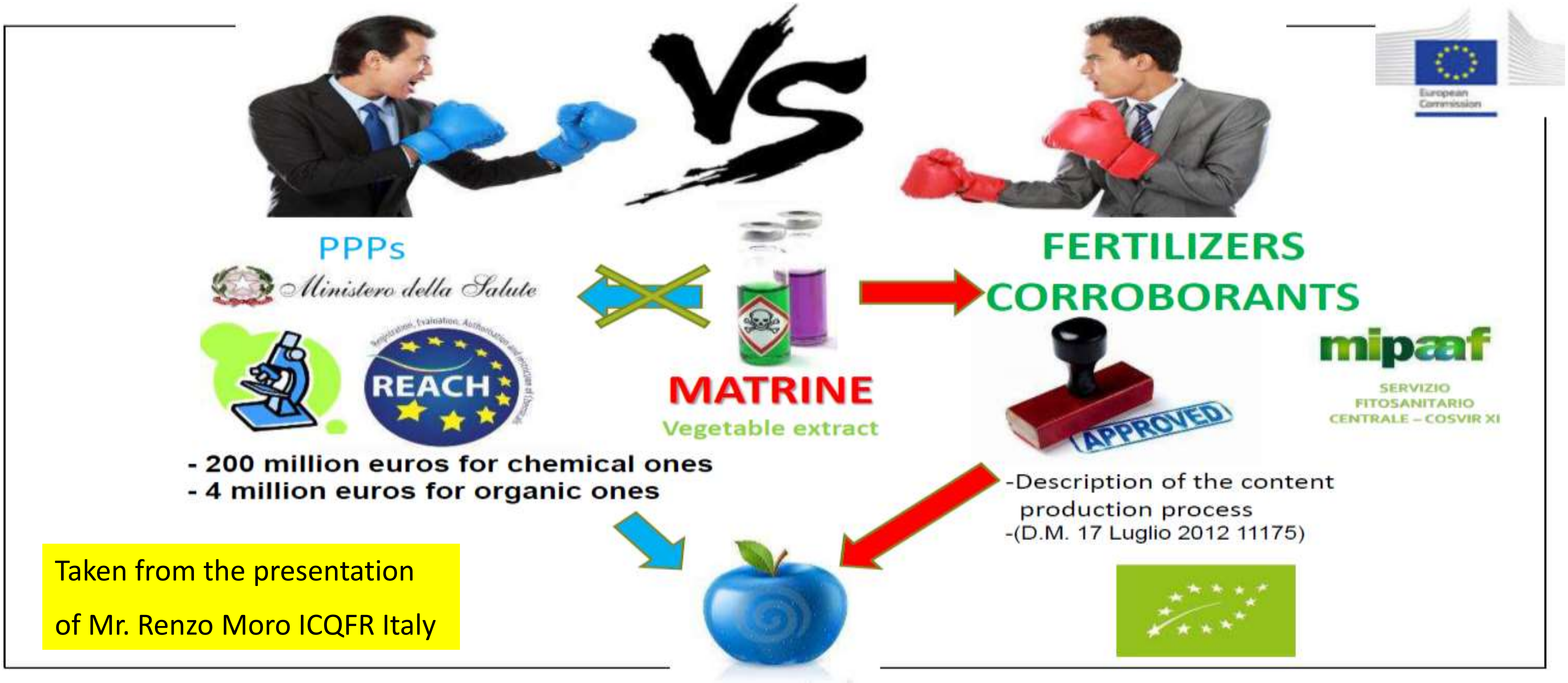
Long-term application and extensive use of synthetic insecticides have resulted in accumulating their residues in food, milk, water, and soil and cause adverse health effects to human and ecosystems. Therefore, application of natural insecticides in agriculture and public health sectors has been increased as alternative to synthetic insecticides. The question here is, are all natural insecticides safe. Therefore, the review presented here focuses on the safety of natural insecticides. Natural insecticides contain chemical, mineral, and biological materials and some products are available commercially, e.g., pyrethrum, neem, spinosad, rotenone, abamectin, *Bacillus thuringiensis* (*Bt*), garlic, cinnamon, pepper, and essential oil products. It can induce hepatotoxicity, renal toxicity, hematotoxicity, reproductive toxicity, neurotoxicity, and oxidative stress. It can induce mutagenicity, genotoxicity, and carcinogenicity in mammals. Some natural insecticides and active compounds from essential oils are classified in categories Ib (Highly hazardous) to U (unlikely toxic). Therefore, the selectivity and safety of natural insecticides not absolute and some natural compounds are toxic and induce adverse effects to experimental animals. In concussion, all natural insecticides are not safe and the term "natural" does not mean that compounds are safe. In this respect, the term "natural" is not synonymous with "organic" and not all-natural insecticide products are acceptable in organic farmers.

# Modern alternative preparations often contain essential oils – some of them haw quite low LD50 values

TABLE 2: Toxicity of some essential oil compounds to experimental animals.

| Compound | Animal | Route | $LD_{50}$ mg/kg. b.wt. | WHO category |
|---|---|---|---|---|
| Category II (Moderately hazardous), 50-2000 mg/kg | | | | |
| Thujone | Mice | Subcutaneous | 87.5 | II |
| Pulegone | Mice | Intraperitoneal | 150 | II |
| 3-Isothujone | Mice | Subcutaneous | 442.2 | II |
| Apiol | Dogs | Intravenous | 500 | II |
| 2-Acetonaphthone | Mice | Oral | 599 | II |
| 2-Methoxyphenol | Rats | Oral | 725 | II |
| Thymol | Rats | Oral | 980 | II |
| Linalool | Rats | Oral | >1000 | II |
| Cinnamaldehyde | Guinea pigs | Oral | 1160 | II |
| Methyl eugenol | Rats | Oral | 1179 | II |
| Dillapiol | Rats | Oral | 1000-1500 | II |
| Anisaldehyde | Rats | Oral | 1510 | II |
| (+) Carvone | Rats | Oral | 1640 | II |
| γ-terpinene | Rats | Oral | 1680 | II |
| Thymol | Mice | Oral | 1800 | II |
| Methyl chavicol | Rats | Oral | 1820 | II |
| Category III (Slightly hazardous), over 2000 mg/kg | | | | |
| trans-Anethole | Rats | Oral | 2090 | III |
| Cinnamaldehyde | Rats | Oral | 2220 | III |
| Maltol | Rats | Oral | 2330 | III |
| 1,8-Cineole | Rats | Oral | 2480 | III |
| Eugenol | Rats | Oral | 2680 | III |
| Menthol | Rats | Oral | 3180 | III |
| Terpinen-4-ol | Rats | Oral | 4300 | III |
| d-Limonene | Rats | Oral | 4600 | III |
| Citral | Rats | Oral | 4960 | III |
| Category U (unlikely to present acute hazard), 5000 mg/kg | | | | |
| Myrcene | Rats | Oral | 5000 | U |

There are many cases of incorporating botanicals in alterative products for which we do not have a complete toxicologically dossier in the EU and we have many cases of withdrawals of preparations from market. Especially problematic are botanicals from many exotic plants grown outside of EU.  Example of criminal level products under official prosecution are products based on MATRINE (extract from plant *Sophora flavescens*).




Taken from the presentation

of Mr. Renzo Moro ICQFR Italy

Example of MATRINE based preparation without MATRINE being mentioned in the declaration

# Matrifruit

**Biodegradable product. Dries and disperses honeydew (psylle, white fly, etc.) and avoids subsequent proliferation of fungi.**

Plant extract + Lysine + Manganese · Mn (0.5%) + Zinc · Zn (1.5%)

## Product characteristics

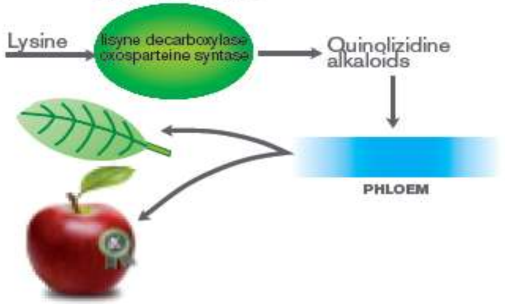
**Matrifruit** is a liquid mixture of plant extract of the *Fabaceae* family, lysine, surfactants, manganese and zinc that can be used to clean honeydew and other debris deposited on leaves and fruits of horticultural crops and fruit trees from petal fall.

Stabilizes and protects complementary treatments with pesticides. **Matrifruit** is recommended to be used in programs to avoid resistance to other products.

**Matrifruit** expresses its activity as a **self-defense response enhancer** of crops, through the following ways:

1) It acts as a surface cleaning detergent of honeydew leaving larvae unprotected and preventing subsequent formation of fungi (sooty mould).
2) It has a barrier effect due to the activity of the plant extract, which reduces or mitigates the attack and damage from some pests.
3) contains lysine, an essential amino acid which is a precursor of the alkaloids of plants that some are able to biosynthesize autoimmune response natural defense against the attack of sucking and eating insects to prevent or delay the damage that can result in crops.

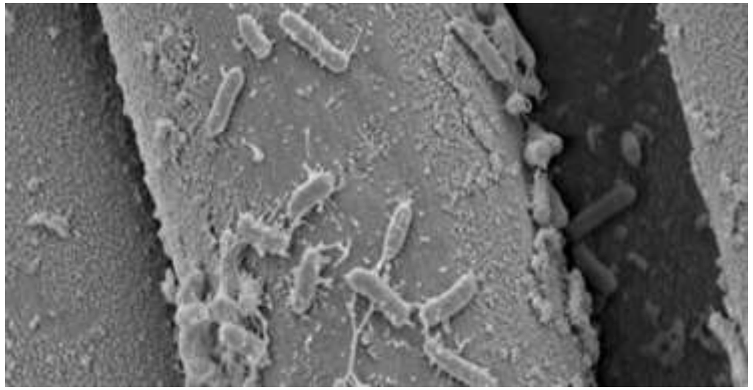
Lysine is a quinolizidine alkaloid precursor, which is produced by plants, for its defense against pests. The lysine transformation to alkaloids, takes place in chloroplasts, then they pass to the phloem and from there to the whole plant resting protected.




### Advantages

- Leaves without honey dew produce more photosynthesis and therefore, the crop yield increases.
- It does not create phytotoxicity.
- There is no safety period after its application.
- No risk to people who apply it.
- No risk to consumers.

## Solutions for...



PROBLEMS with biological contaminants in bacteria-based fungicides and insecticides

During the process of production of bio-fungicides or bio-insecticides, certain species of bacteria which are human pathogens, can develop

- Public health risks of the Bacillus cereus group
- **From 2007 to 2014, EU Member States reported 413 strong-evidence foodborne outbreaks associated with the naturally occurring, soil-borne bacteria Bacillus cereus, which affected 6,657 people and caused 352 hospitalisations.**

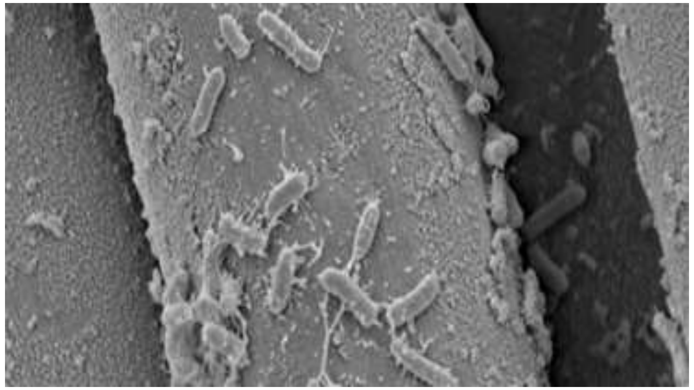
- Bacillus cereus group is very diverse and that there was little information in the literature on other pathogenic Bacillus spp. The Bacillus cereus group comprise eight recognised species and it includes the opportunistic pathogen B. cereus sensu stricto, which is frequently implicated in cases of food poisoning, the entomopathogen B. thuringiensis, from which a number of selected strains are widely used as biopesticides, and the causative agent of anthrax B. anthracis. **B. cereus and B. thuringiensis strains are usually not discriminated in clinical diagnostics or food microbiology**. Thus, the actual contribution of the two species to gastrointestinal and non-gastrointestinal diseases is currently unknown. Most cases of food-borne outbreaks caused by the B. cereus group have been associated with concentrations above 105 CFU/g. The levels of B. cereus that can be considered as a risk for consumers are also valid for B. thuringiensis.

- Several B. thuringiensis strains have been approved as plant protection active substance in Europe and other strains are under evaluation. As no specific Maximum Residue Level (MRL) was fixed for these active substances under Reg. (EC) No 396/2005, the default MRL of 0.01 mg/kg is applicable to all food products. However, this value is currently under discussion at the pesticides residues section of the Standing Committee for Plants, Animals, Food and Feed.

https://kaeltia.com/public-health-risks-of-the-bacillus-cereus-group/

# PROBLEMS with biological contaminants in bacteria-based fungicides and insecticides

## During the process of production of bio-fungicides or bio-insecticides, certain species of bacteria which are human pathogens, can develop



- The EU Biological Hazards (BIOHAZ) Panel recommer
- Obtain information through whole genome sequencing in order to provide unambiguous identification of strains used as biopesticides and assist further safety assessment.
- In cases of food-borne outbreaks associated with the cereus group, characterise strains in detail allowing discrimination of B. thuringiensis from B. cereus, as well as the identification of strains related to commercial B. thuringiensis used as biopesticides.
- Maintain cereus group food-borne outbreak strains in accessible culture collections preferentially managed by reference laboratories.
- Identify markers for commercial thuringiensis strains to allow regular monitoring and easy differentiation in suspect outbreak situations.
- Promote field studies after application of thuringiensis biopesticides in order to inform the possible establishment of pre-harvest intervals.
- Develop research on dose–response and behavioural characteristics of cereus group strains and specifically of B. thuringiensis, to facilitate risk characterisation.
- Develop studies to monitor and characterise the factors that lead to/favour the transfer of the cereus group and specifically B. thuringiensis from the environment to foodstuffs and identify the routes and critical steps of contamination in the food industry.
- For further information, please refer to:
- EFSA Journal, Volume 14, Issue 7, July 2016

https://kaeltia.com/public-health-risks-of-the-bacillus-cereus-group/

- Photo credit: Kanijoman via Foter.com / CC BY

Introduction of MRL (CfU) for microbial residues

Introduction of MRL for microbial metabolites

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 9 June 2016

doi: 10.2903/j.efsa.2016.4524

# Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs

EFSA Panel on Biological Hazards (BIOHAZ)

## Abstract

The *Bacillus cereus* group, also known as *B. cereus sensu lato*, is a subdivision of the *Bacillus* genus that consists of eight formally recognised species: *B. cereus sensu stricto*, *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, *B. cytotoxicus* and *B. toyonensis*. The current taxonomy of the *B. cereus* group and the status of separate species mainly rely on phenotypic characteristics. *Bacillus thuringiensis* strains display a similar repertoire of the potential virulence genes on the chromosome as *B. cereus sensu stricto* strains and it has been shown that these genes can also be actively expressed in *B. thuringiensis* strains. *Bacillus cereus* and *B. thuringiensis* strains are usually not discriminated in clinical diagnostics or food microbiology. Thus, the actual contribution of the two species to gastrointestinal and non-gastrointestinal diseases is currently unknown. Most cases of food-borne outbreaks caused by the *B. cereus* group have been associated with concentrations above $10^5$ CFU/g. However, cases of both emetic and diarrhoeal illness have been reported involving lower levels of *B. cereus*. The levels of *B. cereus* that can be considered as a risk for consumers are also valid for *B. thuringiensis*. There is no evidence that *B. thuringiensis* has the genetic determinants for the emetic toxin cereulide. The Panel has recommended the application of whole genome sequencing to provide unambiguous identification of strains used as biopesticides and the detailed characterisation of outbreak strains allowing discrimination of *B. thuringiensis* from *B. cereus*. Data gaps include: dose–response and behavioural characteristics of *B. cereus* group strains and specifically of *B. thuringiensis*. Field studies after application of *B. thuringiensis* biopesticides are needed to enable the establishment of pre-harvest intervals.



**Keywords:** *Bacillus cereus*, *Bacillus thuringiensis*, food-borne outbreaks, biopesticide



## Generally Recognized as Safe (GRAS) Determination for the Intended Use of *Bacillus subtilis* Strain SG188

**Prepared by:**

Professor Simon M. Cutting

SporeGen Ltd,

Bourne Laboratories,

Royal Holloway University of London,

Egham, UK.



November, 2019

## Relations conventional chemical pesticides / bio-pesticides BP/ biostimulants BS (some experiences from research work)

- In modern plant protection all three groups are closely interconnected and interfere with one another - we also often forget this when preparing dossiers for regulatory purposes, modelling the pesticide fate in the environment

** We have a huge lack in understanding interactions among pesticides, biological control agents and biostimulants

- Typical relations – benefits of use of pesticides and BP and BS together

- 100% dose of pesticide + BP + BS (problems with pesticide efficacy in the beginning of resistance)

- Lowered frequency of use of pesticide + high frequency of use of BP and BS (resistance management, restrictions on accepted NUMBER AND CONCENTRATION of pesticide residues)
- Temporal stop on use of certain active substances to slow down resistance development
- Use of BP and BS for pre-harwest treatments
- Desinfection of soil and equipment

## Relations conventional chemical pesticides / bio-pesticides / biostimulants (some experiences from research work)

- The use of bio-pesticides and biostimulators alters the metabolism of chemical pesticide residues in and on plants.
- In some cases, we accelerate degradation, and in some we slow down degradation.
- The use of bio-pesticides and biostimulators alters the metabolism of the microbe community in and on plants. Bacto- and mycotoxin production can be increased as a response to bio-pesticide induced stress.

**Practical example – increased metal intake in case of seaweed application:**

- Spraying tomato with copper hydroxide based fungicide and seaweed based biostimulant
- 30% increase in intake of copper due to mixing of  fungicide and seaweed biostimulant

Seaweed proteins are chelating agents and have a carrier effect


- Coper content in fruit

when applying  Cu fungicide 6 times 500 g Cu++/ha — 1,97   mg Cu/kg

- Copper content in fruit

when applying  Cu fungicide 6 times 500 g Cu++/ha

+ 6 times seaweed  Ascophyllum  3 kg/ha — 2,57  mg Cu/kg

Seaweed preparation was checked to be almost completly free of copper.

# Practical example – increased pesticide degradation:

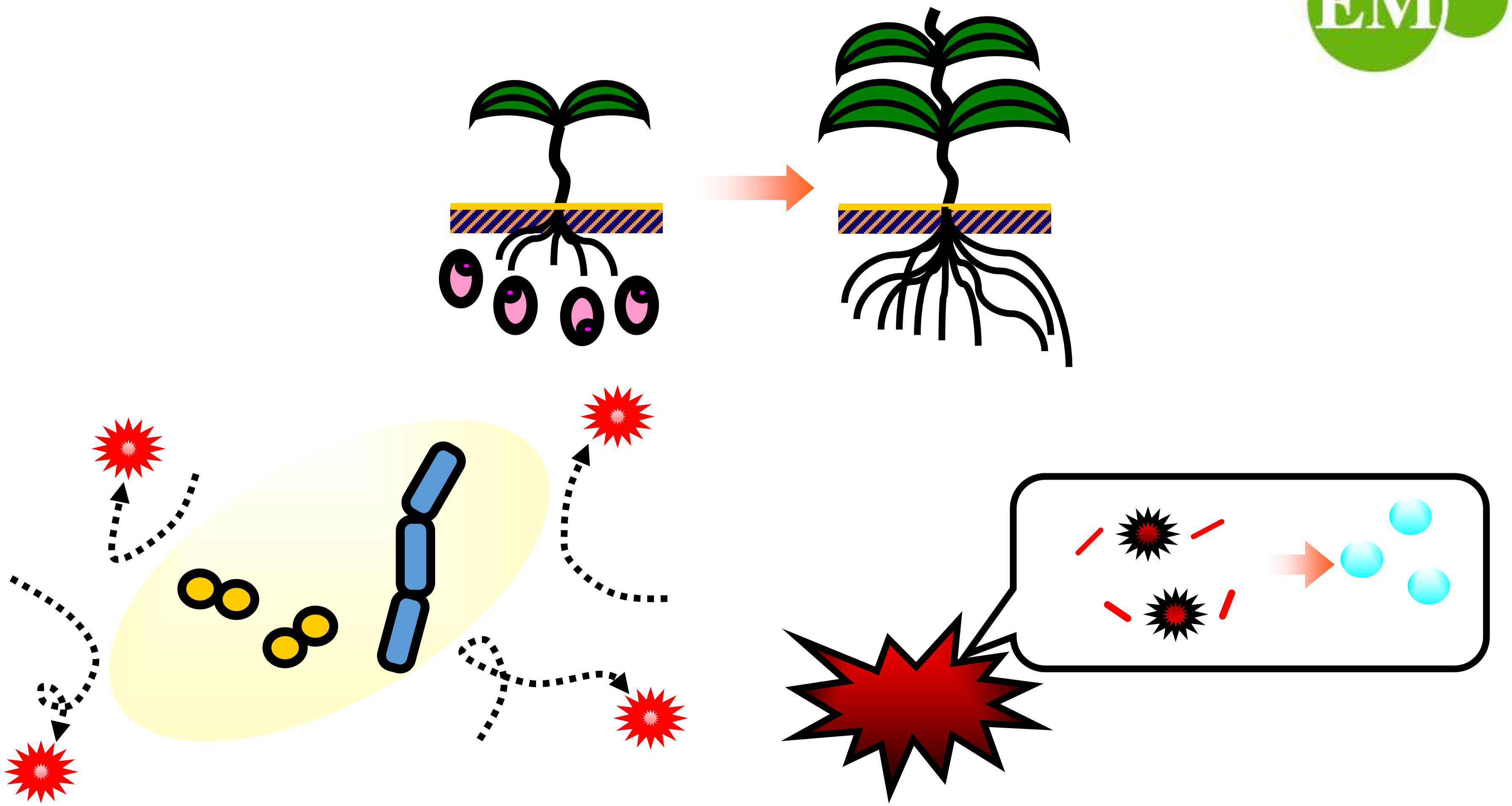
Spraying apples with standard conventional pesticides and EM biostimulant + *Bacillus subtilis* product 4 times prior to harvest in one week intervals

EM – mixture of yeast, different microalgae, phototrophic bacteria and other microorganisms

- >40% increase in degradation of many pesticide residues
- Pesticide content in apples at harvest:

| Pesticide (applied x times a season at recommended dose from the lable) | Residues in standard spry program (mg/kg apples) | Residues in standard spry program + 4 times (EM + Serenade) (mg/kg apples) reduction rate | |
|---|---|---|---|
| Captan 8x | 0,190 A | 0,09 B | -52,6 % |
| Dithianon 4x | 0,170 A | 0,016 B | -90,6 % |
| Trifloxistrobine 2x | 0,090 A | 0,100 A | +11,1 % |
| Pyrimethanil 2 x | 0,070 A | 0,06 A | -14,3 % |
| Difenconazole 2x | 0,008 A | 0,009 A | +12,5 % |
| Fluxapyroxad 2x | 0,020 A | 0,012 B | -40,0 % |
| Fluopyram 2x | 0,022 A | 0,012 B | -45,5 % |
| Chloranthraniliprol 2x | 0,037 A | 0,033 A | -10,8 % |
| Spirotetramat 1x | 0,011 A | 0,011 A | 0,0 % |

# 2.4. EM POWER



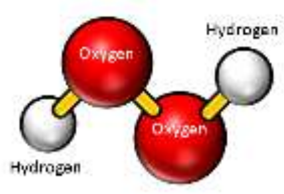
Lactic acid bacteria + phototropic bacteria + yeast

**Thanks to the formula, EM POWER can be produced.**

# Practical example – increased pesticide degradation:

Spraying apples with standard conventional pesticides and H2O2 product

4 times prior to harvest in one week intervals (0,18 % H2O2 / 1000 l/ha)



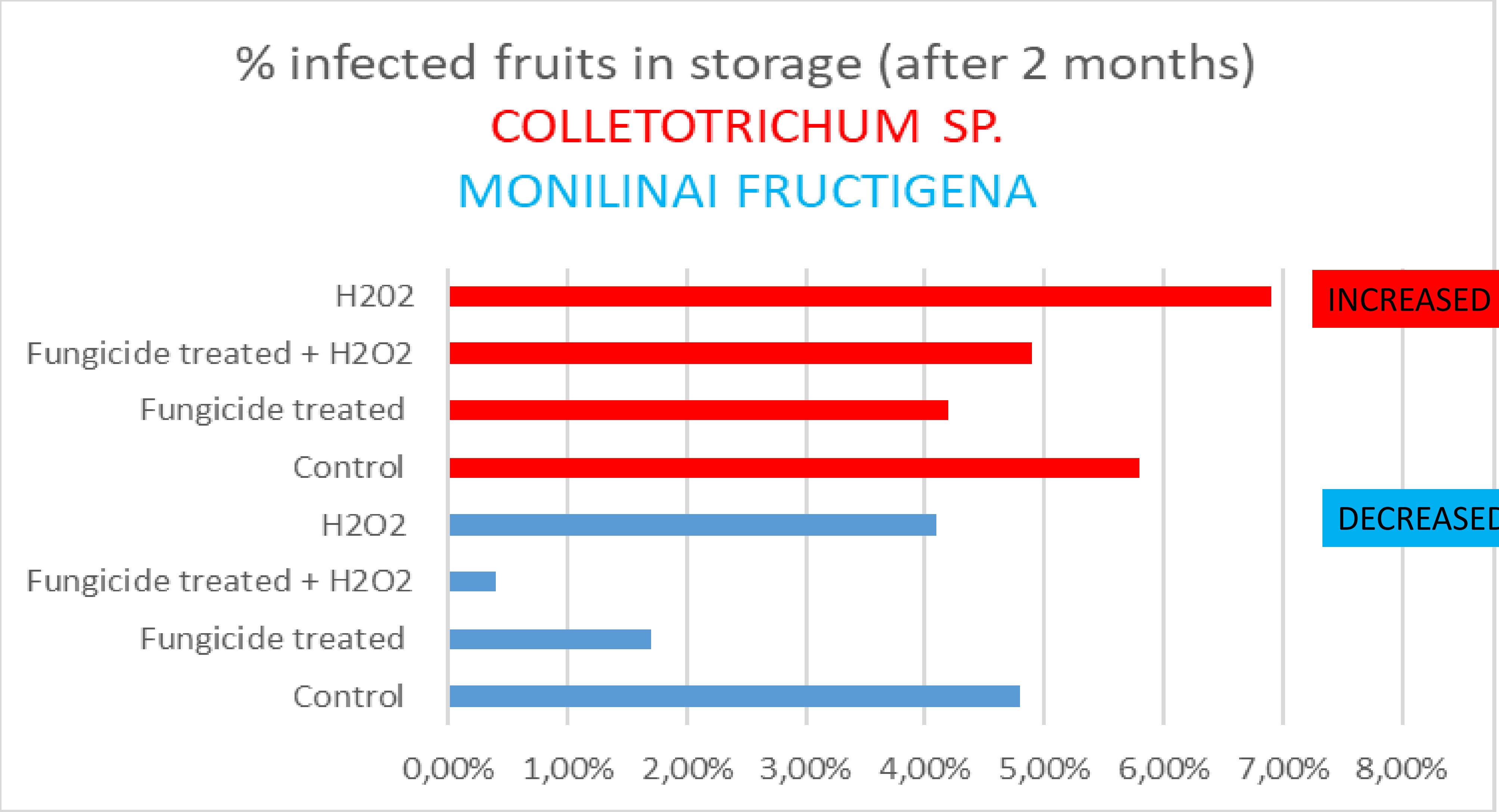

- >20 % increase in degradation at some pesticide residues
- Pesticide  content in apples at harvest:

| Pesticide (applied x times a season at recommended dose from the lable) | Residues in standard spry program (mg/kg apples) | Residues in standard spry program + 4 times (H202) (mg/kg apples) | REDUCTION RATE |
|---|---|---|---|
| Captan 8x | 0,190 A | 0,140 A | - 26,3 % |
| Dithianon 4x | 0,170 A | 0,160 A | - 5,9 % |
| Trifloxistrobine 2x | 0,090 A | 0,080 B | -11,1 5 |
| Pyrimethanil 2 x | 0,070 A | 0,070 A | 0,0 % |
| Difenconazole 2x | 0,008 A | 0,009 A | +12,5 % |
| Fluxapyroxad 2x | 0,020 A | 0,013 B | - 35,0 % |
| Fluopyram 2x | 0,022 A | 0,018 B | -18,2 % |
| Chloranthraniliprol 2x | 0,037 A | 0,037 A | 0,0 % |
| Spirotetramat 1x | 0,011 A | 0,012 A | +9,1 % |

**Practical example – EFFECT ON FUNGI CAUSING STORAGE FRUIT DECAY:**

Spraying apples with standard conventional pesticides and a H2O2 product
4 times prior to harvest in one week intervals (0,18 % H2O2 / 1000 l/ha)

CULTIVAR PINOVA

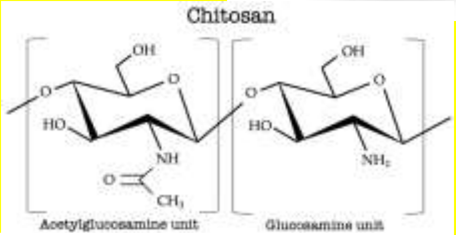



INCREASED INFESTATION

DECREASED INFESTATION

# Practical example – decreased pesticide degradation:

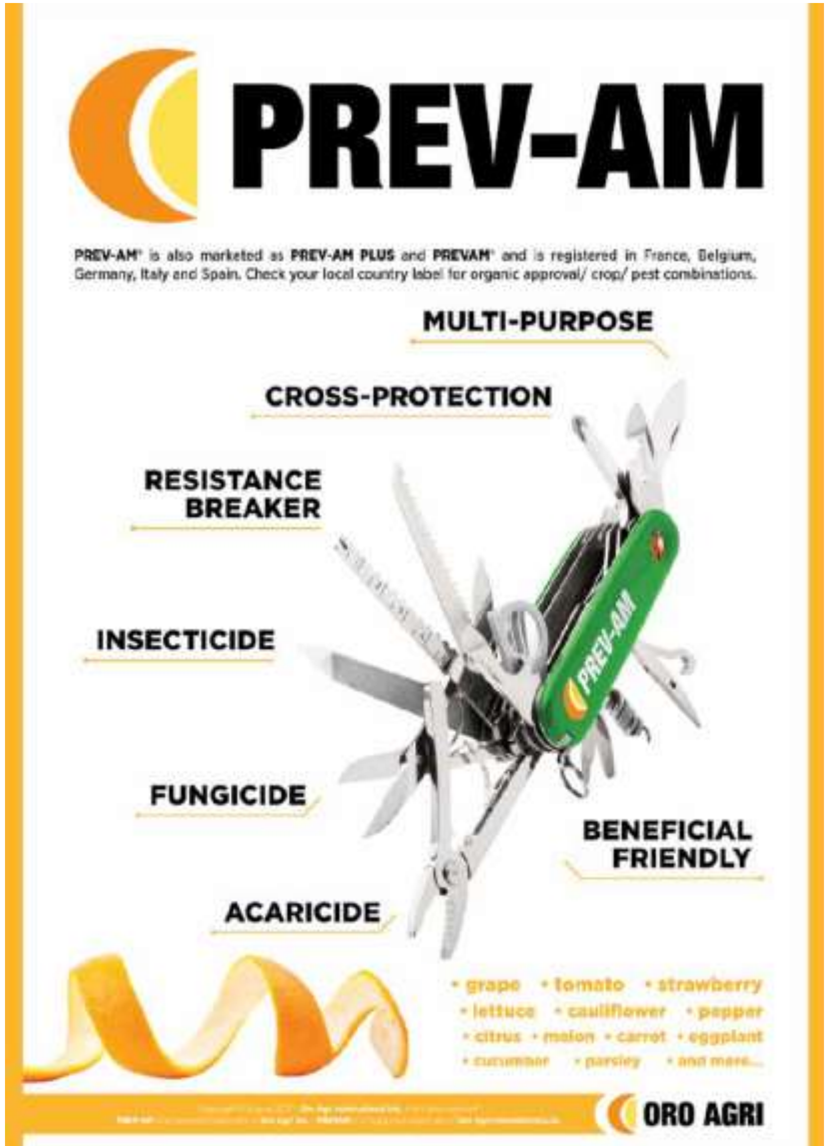
- Spraying apples with standard conventional pesticides and chitosan products 4 times a season prior to harvest in 2-week intervals
- 25 % decrease in degradation of certain pesticide resideus




## Pesticide content in apples at harvest:

| Pesticide (applied x times a season at recommended dose from the lable) | Residues in standard spry program (mg/kg apples) | Residues in standard spry program + 4 times Chitobasic 3 kg/ha (mg/kg apples) | |
|---|---|---|---|
| Captan 8x | 0,190 A | 0,201 A | + 5,8 % |
| Dithianon 4x | 0,170 A | 0,199 A | + 17,1 % |
| Trifloxistrobine 2x | 0,090 A | 0,112 B | + 24,4 % |
| Pyrimethanil 2 x | 0,070 A | 0,09 B | + 28,6 % |
| Difenconazole 2x | 0,008 A | 0,007 A | -12,5 % |
| Fluxapyroxad 2x | 0,020 A | 0,027 B | + 35,0 % |
| Fluopyram 2x | 0,022 A | 0,028 B | + 27,3 % |
| Chloranthraniliprol 2x | 0,037 A | 0,039 A | + 5,4 % |
| Spirotetramat 1x | 0,011 A | 0,013 A | + 18,2 % |

Similar results as with CHITOSAN were obtained in the case of very frequent use of orange based essential oils and kaolin clay




Pictures taken from internet

Practical example: use of detergents Fuji apple (against Eriosoma lanigerum)
Apples treated with standard pesticides and not treated with detergent
Apples treated with standard pesticides and at the end of the season threated with LDC detergent 2 times at a dose of 4 l/ha (once 3 weeks and once 1 week prior harvest)
Apples treated with standard pesticides and at the end of the season threated with LDC detergent 4 times at a dose of 4 l/ha (once 6, 5, 4 and 3 weeks prior the harvest)


| Pesticide (applied x times a season at recommended dose from the lable) | Residues in standard spry program mg/kg | Residues in standard spry program + 2 X LDC det. (mg/kg) | | Residues in standard spry program + 4 X LDC det. (mg/kg) | REDUCTION RATE |
|---|---|---|---|---|---|
| Captan 7x | 0,115 A | 0,102 A | -11,3 % | 0,08 B | -30,4 % |
| Dithianon 4x | 0,090 A | 0,067 A | -25,6 % | 0,012 B | -86,7 % |
| Trifloxistrobine 2x | 0,040 A | 0,038 A | -5,0 % | 0,023 B | -42,5 % |
| Pyrimethanil 2 x | 0,060 A | 0,07 A | 16,7 % | 0,05 A | -16,7 % |
| Difenconazole 3x | 0,012 A | 0,011 A | -8,3 % | 0,003 B | -75,0 % |
| Fluxapyroxad 2x | 0,080 A | 0,072 A | -10,0 % | 0,011 B | -86,3 % |
| Fluopyram 2x | 0,025 A | 0,022 A | -12,0 % | 0,009 B | -64,0 % |
| Chloranthraniliprol 2x | 0,034 A | 0,033 A | -2,9 % | 0,012 B | -64,7 % |
| Spirotetramat 2x | 0,016 A | 0,014 A | -12,5 % | 0,009 B | -43,8 % |
| | | | | | |

# A New View of Sooty Blotch and Flyspeck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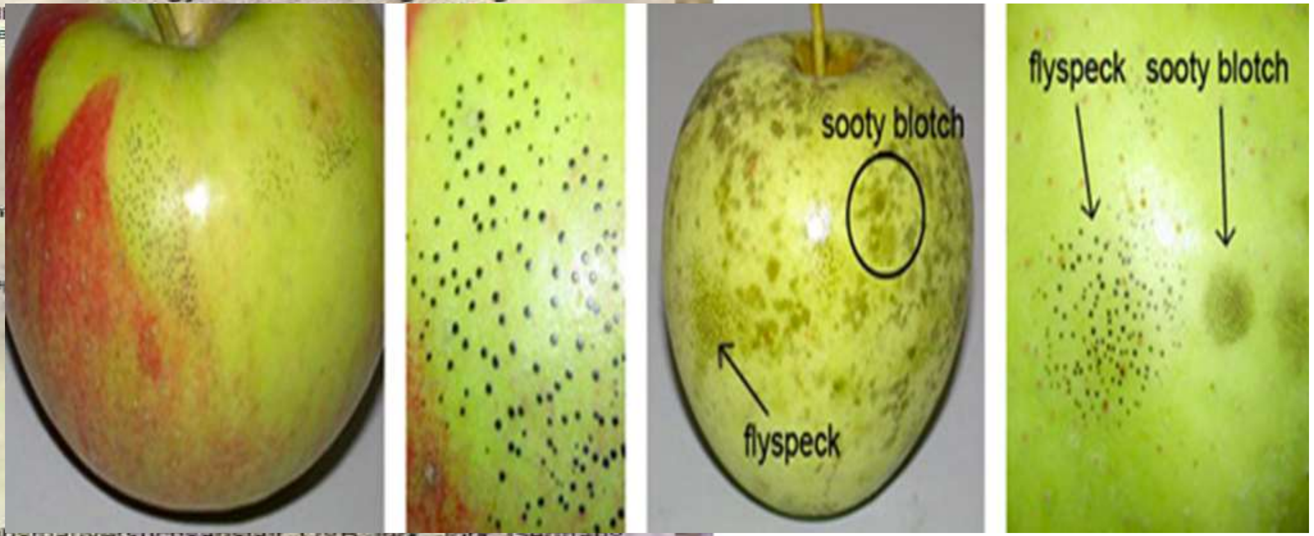
INRES/Gartenbauwissenschaften, Univer
Bonn, Germany


Sooty blotch and flyspeck (SBFS) fungi colonize the surface wax layer of the fruit of apple, pear, persimmon, banana, orange, papaya, and several other cultivated tree and vine crops. Because their hyphae, fruiting bodies, and survival structures are melanized (darkly pigmented), SBFS colonies appear as blemishes (Fig. 1). The disease occurs worldwide in regions with moist growing seasons. In addition to cultivated fruit crops, SBFS fungi also grow on the surfaces of stems, twigs, leaves, and fruit of a wide range of wild plants.

SBFS fungi cause no physiological damage to the underlying fruit except an accelerated desiccation of apples during cold storage, presumably due to a damaged wax layer. Nevertheless, SBFS is regarded as a serious disease by fruit farmers and plant pathologists because it can cause substantial economic damage. The smudges and stipples of SBFS often result in downgrading of fruit from premium fresh-market grade to processing use. In eastern North America, high-value apple cultivars can lose as much as 90% of their value in this way. Even when relatively few fruit in an orchard block are blemished, it may not be cost effective to sort them out manually, so entire harvests must be diverted to processing (23,24). Economic damage from SBFS is not limited to North America; losses from SBFS blemishing of apple, pear, persimmon, hawthorn, and other fruit crops occur worldwide. Since economic losses are most common on apple, nearly all of the research on the SBFS complex has focused on this crop.

Eleven years ago, Williamson and Sutton (70) reviewed the etiology, biology, and control of SBFS on apple. Their article remains the only prior review, although SBFS fungi have been studied for nearly 180 years. The present update describes the major shifts that have occurred during the past deca
ing the genetic diversity of the SBFS complex,
geography and environmental biology, and dev
management strategies.


Corresponding author: Mark L. Gleason, E-mail: mgleason@iastate.edu


## Taxonomy, Diversity, Biogeography, and

**Taxonomy.** Reliable identification of SBFS
many generations of mycologists. In general, the
lenging to isolate and grow in pure culture. Th
dinarily slowly, so are easily overgrown on aga
phytes. Surface disinfection of the fruit before isolation is not helpful since the epiphytic SBFS fungi are killed as readily as non-SBFS epiphytes. The fact that many SBFS species sporulate rarely or not at all, either on fruit or in culture, frustrates morphological description of species. To compound the problem, colony morphology of an SBFS isolate on fruit can differ radically from that on agar media and varies considerably on media with changes in pH,

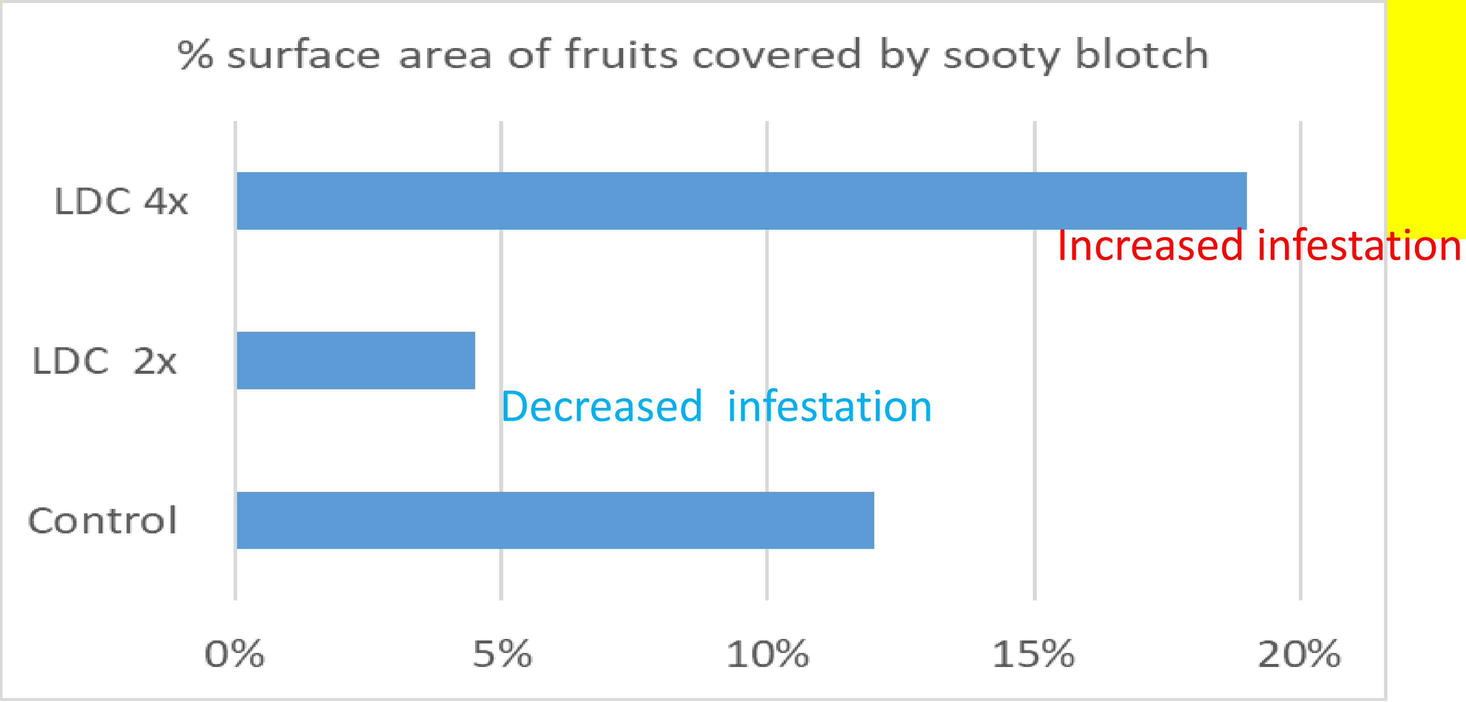
New problems in the field of mycotoxins in fruit

- **Practical example: use of detergents Fuji apple (against Eriosoma lanigerum)**

a) Apples treated with standard pesticides and not treated with detergent

b) Apples treated with standard pesticides  and at the end of the season threated with LDC detergent 2 times  at dose  4 l/ha (once 3 weeks and once 1 week prior harvest)

c) Apples treated with standard pesticides  and at the end of season threated with LDC detergent 4 times  at dose  4 l/ha (once 6, 5, 4 and 3  weeks prior the harvest)




Use of detergents can influence population dynamics of sooty blotch fungi significantly – effects on structure and composition of fruit skin

Apple sooty blotch fungi are sources of mycotoxins

Fungi developed after treatment with high doses of detergent

Detergent damaged the fruit skin



FOOD CHEMISTRY



## Production of Trichothecenes by the Apple Sooty Blotch Fungus *Microcyclospora tardicrescens*

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RIS Citation GO

### Abstract


The sooty blotch and flyspeck (SBFS) syndrome of apples and other fruits is caused by a complex consortium of epiphytic fungi that colonize the fruit cuticula. SBFS fungal strains isolated from apples were screened for growth inhibition of the phytopathogen *Colletotrichum fioriniae* in dual culture tests. Extracts of 11 isolates of SBFS fungi (*Microcyclospora malicola*, *Microcyclospora pomicola*, *Microcyclospora tardicrescens*, and *Microcyclosporella mali*) inhibited growth of the test strains and were studied for production of antibiotics. A strain of *Microcyclospora tardicrescens* strongly inhibited growth and was cultivated on a larger scale to characterize its secondary metabolites. Bioassay-guided fractionation and subsequent structure elucidation by spectroscopic and spectrometric methods (NMR, HRMS) yielded trichothecolone acetate (**1**) and its novel derivative (*S*)-7-hydroxytrichothecolone acetate (**2**) as active principles. *Microcyclospora tardicrescens* was thus identified as a producer of the hazardous trichothecene type mycotoxins for the first time, which should give reason to monitor these foodborne fungi more carefully in the future.

**Keywords: microbial interactions; secondary metabolites; sooty blotch and flyspeck; structure elucidation; trichothecolone**

# Practical example: Interference among fungicides and bio-protectants in control of wheat *Fusarium* disease

a) Azoxystrobin 1x BBCH45

b) Göemar (*Ascophyllum*) + Trifender (*Trichoderma*)) 1x BBCH35 + Azoxystrobin 1x BBCH45

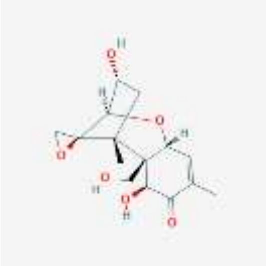
c) Azoxystrobin 1x BBCH35 + (benzovindiflupyr + prothioconazole) 1x BBCH59

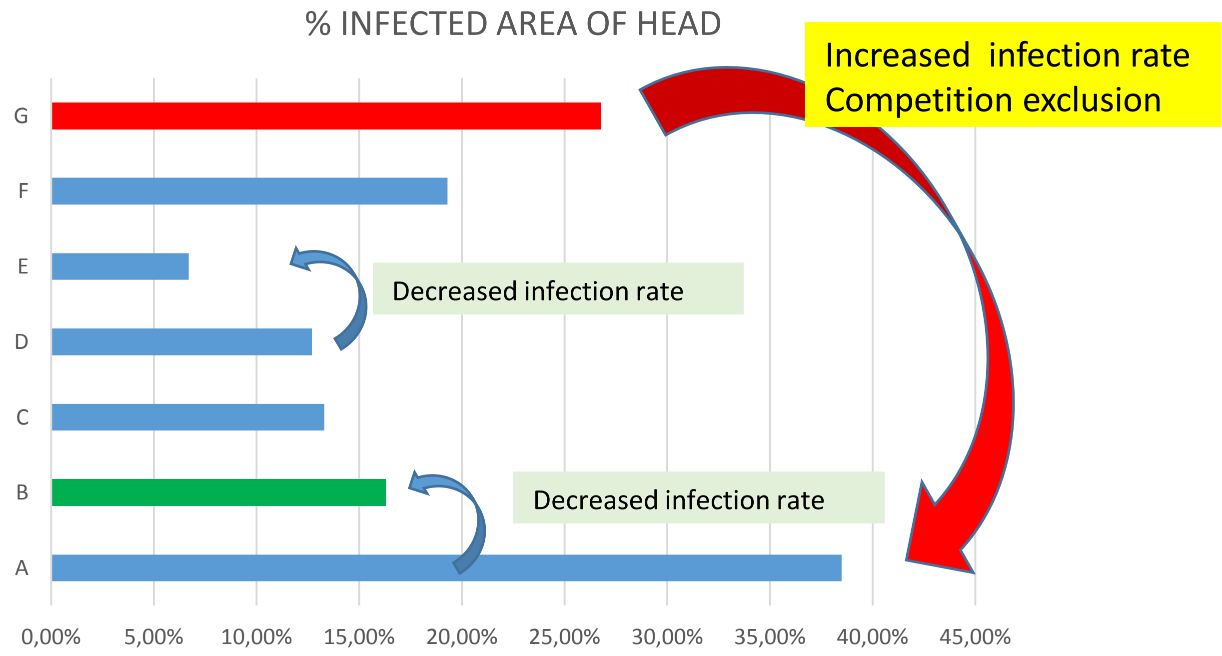
d) (benzovindiflupir + protiokonazol) 1x BBCH55

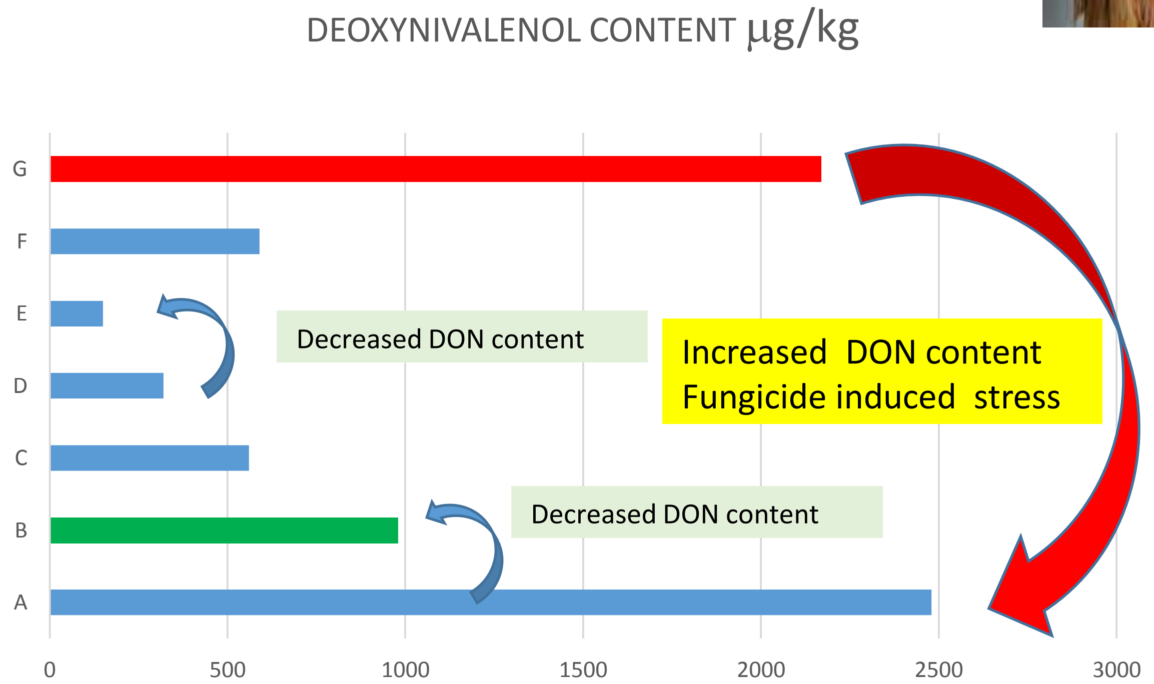
e) (azoxystrobin + Göemar (*Ascophyllum*) + Trifender (*Trichoderma*)) 1 x BBCH35 + (benzovindiflupyr + prothioconazole) 1x BBCH 59

f) Göemar (*Ascophyllum*) + Trifender (*Trichoderma*) 1 x BBCH35 + 1 x BBCH 59

g) control not treated





## Practical example: Interference among fungicides and bio-protectants in control of wheat *Fusarium* disease

a) Azoxystrobin 1x BBCH45

b) Göemar (*Ascophyllum*) + Trifender (*Trichoderma*)) 1x BBCH35 + Azoxystrobin 1x BBCH45

c) Azoxystrobin 1x BBCH35 + (benzovindiflupyr + prothioconazole) 1x BBCH59

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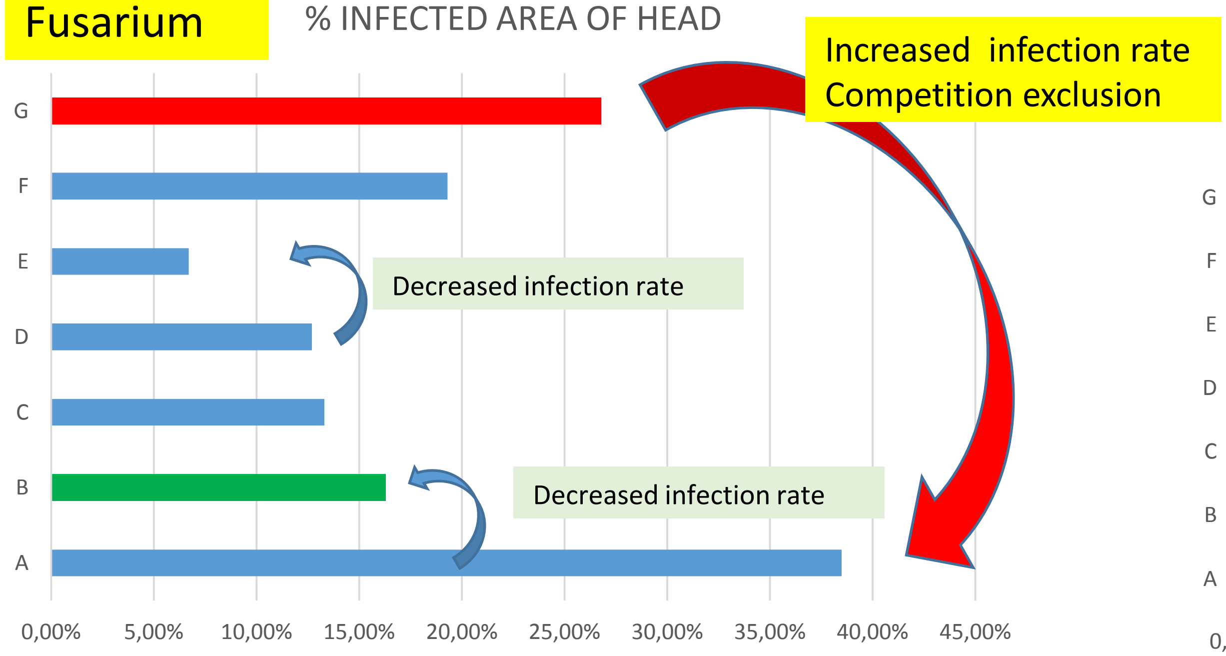
f) Göemar (*Ascophyllum*) + Trifender (*Trichoderma*) 1 x BBCH35 + 1 x BBCH 59

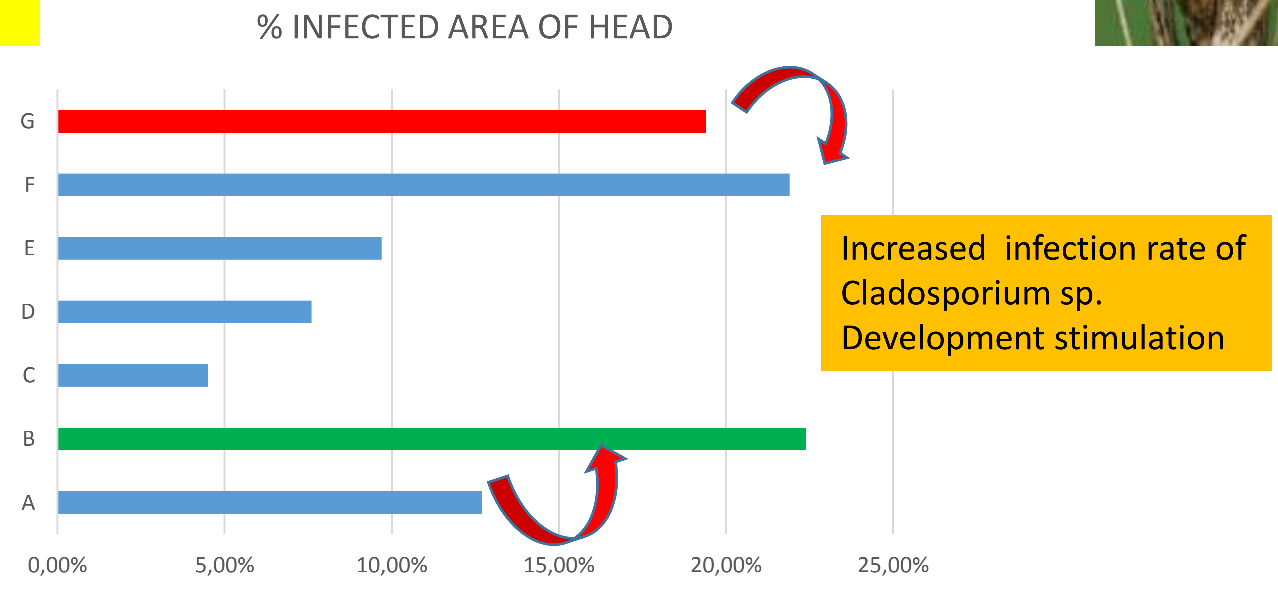
g) control not treated

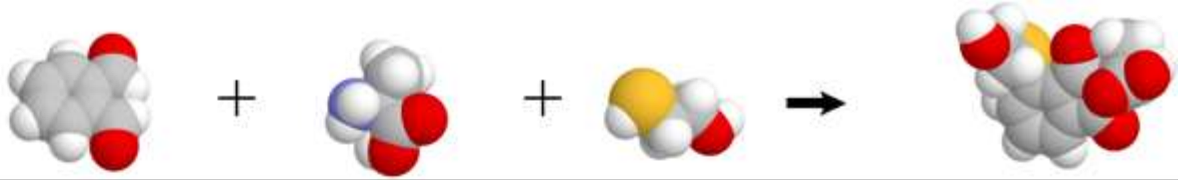
Interaction with Cladosporium sp.
Cladosporin toxins ???
Antifungal agents – human toxicants?

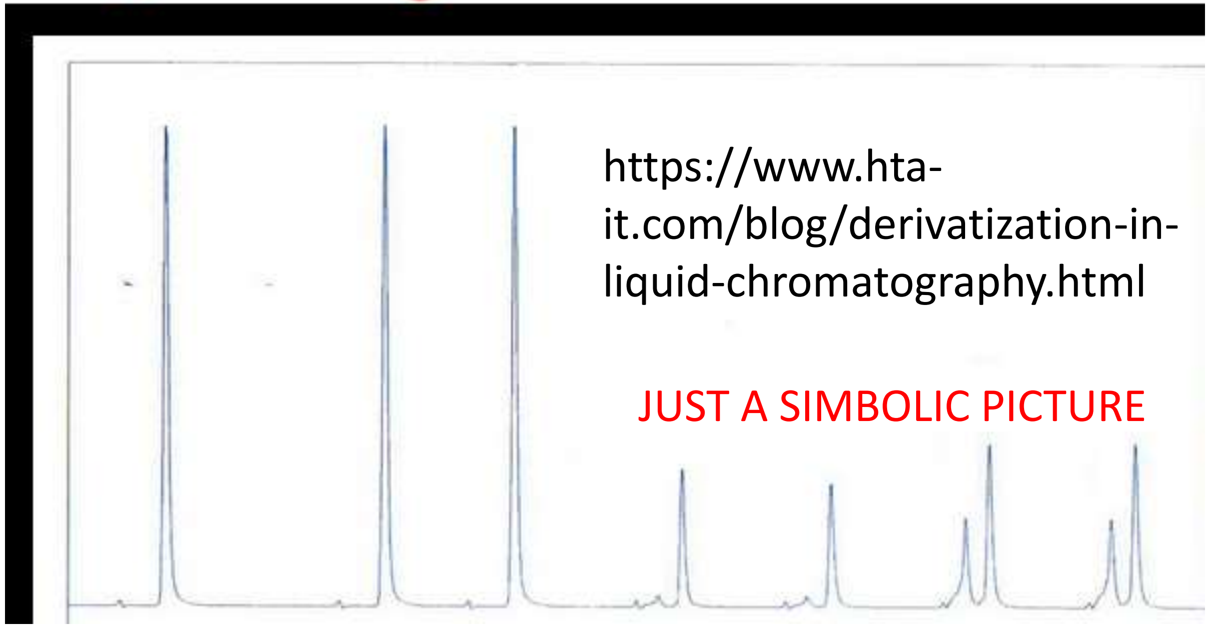
Fusarium

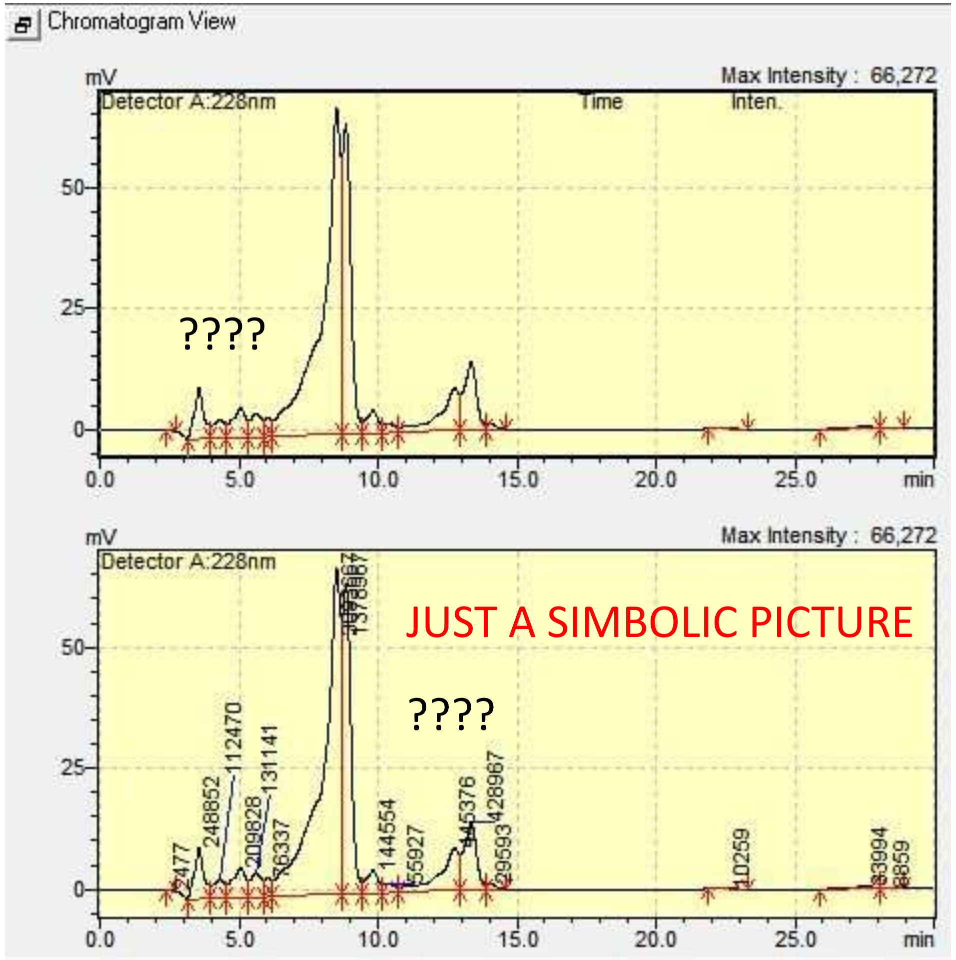



Cladosporium sp.





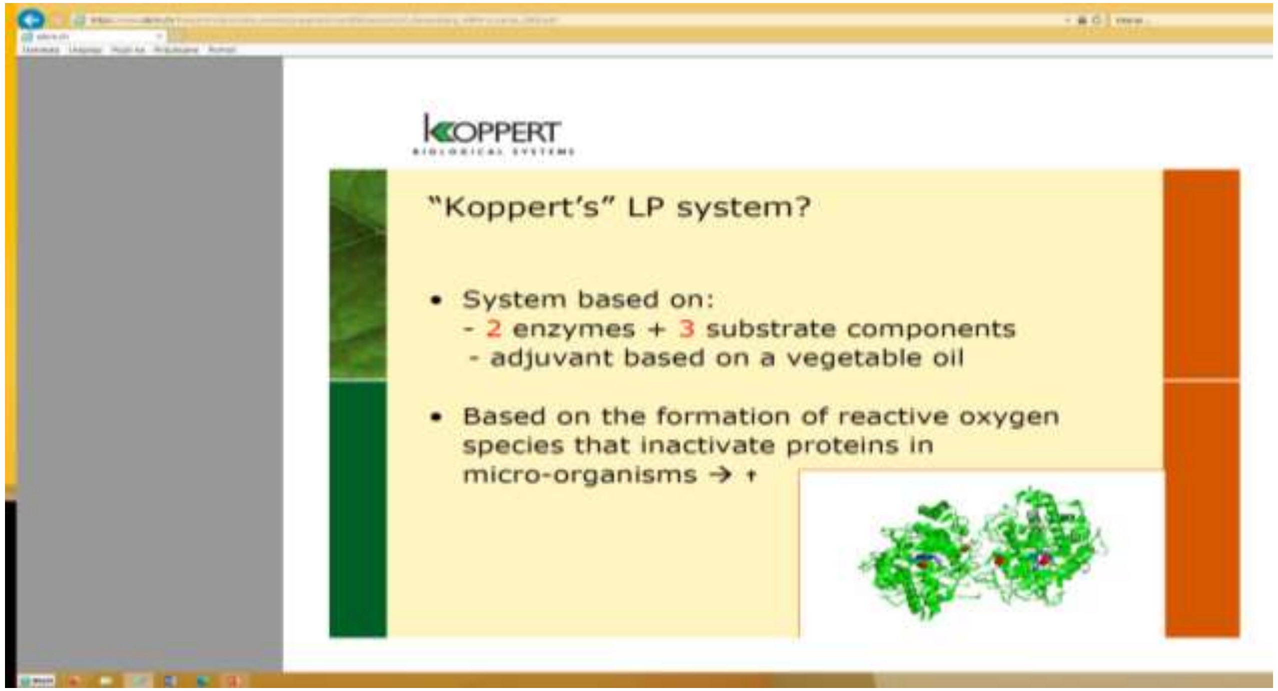



https://www.researchgate.net/topic/HPLC-Analysis

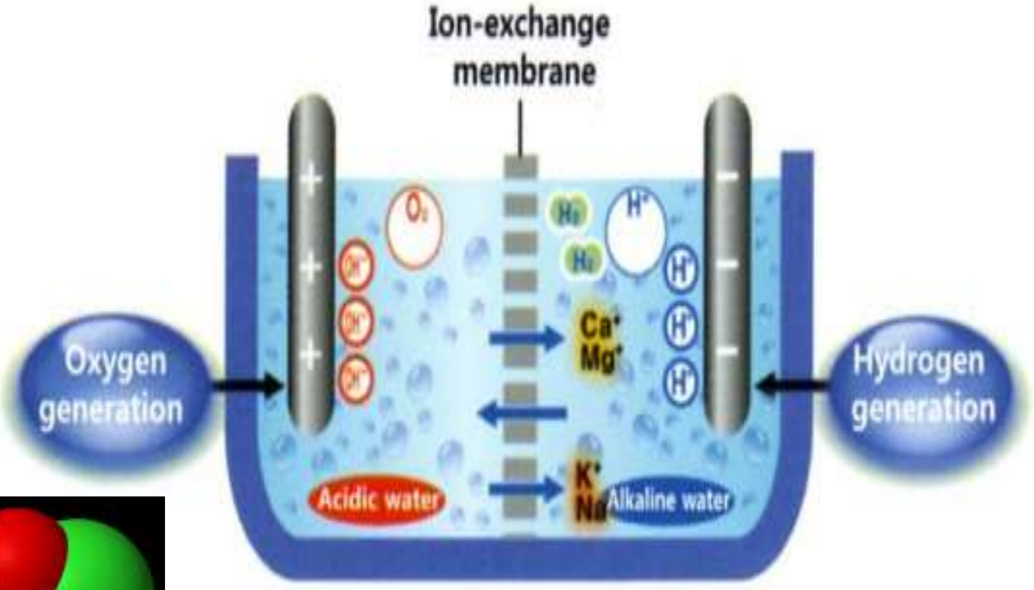
**In laboratory analysis of pesticide residues a lot of unknown substances are detected in samples from plants treated with preparations with peroxidase action**

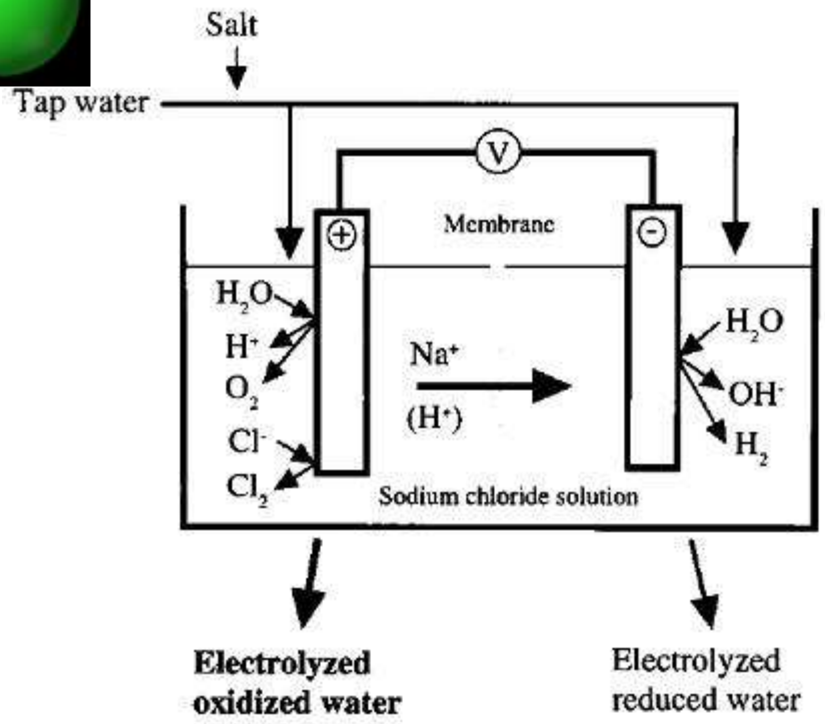
1) Titanium based biostimulants
2) Laktoperoxidase based biostimulants or bio-pesticides
3) H2O2 based biostimulants or bio-pesticides
4) Use of electrolized water


Enzicur, a novel fungicide against powdery mildew, based on the Lactoperoxidase system (LPS). Foreign TitleEnzicur, un nouveau fongicide contre l'oidium, a base du systeme Lactoperoxydase (LPS).Piron, M.; Ravensberg, W.; Hora, K.Journal 10e Conférence Internationale sur les Maladies des Plantes, Tours, France, 3, 4 & 5 Décembre, 2012 2012 pp. 620-629 Record Number20133118214

ENZICUR is a product registered in the Netherlands in 2007. The registration file was submitted in France in april 2010. It contains two active substances: potassium iodide and potassium thiocyanate. This **new natural fungicide** has been developed on the basis of the so-called Lactoperoxidase (LP)-system, an anti-microbial system active in bovine milk. The activity of the LP-system is the enzymatic formation of reactive oxygen molecules which react with proteins in micro-organisms, such as bacteria and fungi. ENZICUR is a curative contact fungicide and devlopped to control powdery mildew in greenhouse crops. After the description of the LP System and the mode of action, its efficacy against powdery mildew in strawberry, cucumber and tomato will be proven.


What kind of product is electrolized water ????
Pesticide / biostimulant

## How can sodium hypochlorite be produced?

This overall chemical reaction can be expressed as follows:
Salt + Water + Energy ⟶ Sodium hypochlorite + Hydrogen
$NaCl + H_2O + 2e \longrightarrow NaClO + H_2$

Sodium hypochlorite can be produced in two ways:
- By dissolving salt in softened water, which results in a concentrated brine solution. The solution is electrolyzed and forms a sodium hypochlorite solution in water. This solution contains 150 g active chlorine ($Cl_2$) per liter. During this reaction the explosive hydrogen gas is also formed.

For on-site salt electrolysis, a solution of salt (NaCl) in water is applied. Sodium ($Na^+$) and chloride ($Cl^-$) ions are produced.
$4NaCl^- \rightarrow 4Na^+ + 4Cl^-$

By leading the salty solution over an electrolysis cell, the following reactions take place at the electrodes:
$2Cl- \rightarrow Cl_2 + 2e- \; 2H_2O + 2e- \rightarrow H_2 + 20H^-$
$2H_20 \rightarrow O_2 + 4H^+ + 4e-$

Subsequently, chlorine and hydroxide react to form hypochlorite:
$OH^- + Cl_2 \rightarrow HOCl + Cl^-$

By adding hypochlorite to water, hypochlorous acid (HOCl) is formed:
$NaOCl + H_2O \rightarrow HOCl + NaOH^-$

Hypochlorous acid is divided into hydrochloric acid (HCl) and oxygen (O). The oxygen atom is a very strong oxidator.

Some comments:
http://www.chem1.com/CQ/ionbunk.html

**"Electrolyzed oxidizing water" ("EO" water)**

- This term is commonly applied to the products of "water ionizing" machines when the marketing focus is on bactericidal properties, rather than on the false claims about the health benefits of alkaline drinking water.
- As is explained above, these electrolysis devices produce what amounts to a dilute solution of sodium hypochlorite, similar to what can be obtained by diluting some ordinary laundry bleach such as Clorox to the point at which the odor is no longer noticeable. If this is made slightly acidic (by addition of some vinegar or lemon juice, for example), then most of the hypochlorite ion is in the form of hypochlorous acid, which is a bactericide and is the active product produced when chlorine is used to disinfect drinking water.
- The only real issues here are
- Is it worth purchasing an expensive electrolysis device to generate the same mixture than one can get perhaps several hundred gallons of by diluting a $1.49 bottle of home laundry bleach?
- Is this stuff any more effective for purposes such as disinfecting vegetables and foods than by simply washing with ordinary water, or with water acidified by vinegar or lemon juice?
- Do you really want your food to come into contact with an oxidizing agent that can react with some of the organic components to produce potentially carcinogenic by-products? (This is, of course, one argument against the use of chlorine to disinfect waters containing a lot of organic material)
- So while "EOW" may have some legitimacy as a disinfectant, I consider it somewhat deceptive when promoters tout it (as some do) as a special, "chemical-free" disinfectant. See also this *Food Quality* article.

## Problems of spoilage of microbial based biological products and biostimulants

Microbial threats CAN APEAR  during improper storage of microbial based products (many time called tonic) and human pathogens can develop inside a package which is left open for too long period of time.

Many times on products label a highest number of treatments is not defined and waiting periods are not clearly defined. It is not advised to spend whole amount of product once package is opened.

**Improvements are need in defining of more detailed advice about use and handling  of biological products.**

# Conclusion

- We urgently need many new biological agents (BA) and biostimulants (BS) to significantly reduce use of conventional chemical pesticides

- Toxicological burden to human population end environment could be significantly reduced by introduction of new alternative products but not completely

- Interactive use of pesticides with BA and BS  in IPM based plant protection brings many interactions among conventional pesticides and biological pesticides and biostimulants what alters pesticide environmental fate, plant metabolism and plant and human microbiome metabolism

- We need to keep in mind that many ways of toxic effects of bio-protectants exist which we do not undusted completely and therefore we shall not accept oversimplified procedures for biological pesticide and biostimulant registration

**"There is no such thing as a completely safe plant protection product"**

Harmless sticky trap for insect monitoring could be a deadly trap for birds

INTERNATIONAL YEAR OF
PLANT HEALTH
2020

Thank you for your attention!

# Conclusion II

- Algae based and protein based products can contain substances that can complex with pesticides and cause carrier effects – increased intake of pesticide active substance in plant.

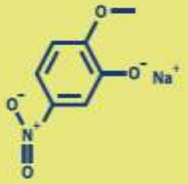
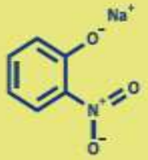
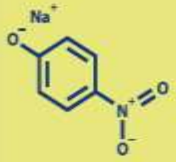
- Algae based and protein based products can in case of very frequent application form a layer reach on nutrients on surface of fruits and vegetables which can serve as grooving substratum for certain saprophytes that produce substances harmful to humans.

- Very frequent use of biostumulants can cause overproduction of antinutritional agents, phytoalexins and hormone-acting substances in plants what make plants less suitable for consumption in terms of human health (people with specific types of health disorders).

- Use of bio stimulants which action is based on peroxidase effect can cause production of reactive pesticide metabolites for which we do not have a clear toxicological profile.

Drinking water in the middle of modern orchard frequently treated with pesticides (bio-pesticides ???)

# **Active** substances

| Common name | Sodium 5-nitroguaiacolate | Sodium o-nitrophenolate | Sodium p-nitrophenolate |
|---|---|---|---|
| Chemical name | Sodium 2-methoxy-5--nitrophenolate | Sodium 2-nitrophenolate | Sodium 4-nitrophenolate |
| CAS No | 67233-85-6 | 824-39-5 | 824-78-2 |
| Molecular formula | $C_7H_6NNaO_4$ | $C_6H_4NNaO_3$ | $C_6H_4NNaO_3$ |
| Molecular mass | 191.1 g/mol | 161.1 g/mol | 161.1 g/mol |
| Structural formula |  |  |  |

# **Physical and Chemical** properties

| | |
|---|---|
| Appearance | Brown yellow liquid |
| pH | 8.36 at 21°C |
| Explosives properties | Risks of explosion almost none in the recommended conditions of storage. Real risk in case of fire or accumulation of the emanations |
| Relative density | 1 about |
| - Water | Miscible with water in all proportions. Gives limpid solution. |
| - Organic solvents | Non miscible with almost all organic solvents. |

Atonik

# **Toxicological** information

| Acute toxicity | |
|---|---|
| Ingestion (rat) | LD50 > 2000 mg/kg |
| Skin contact (rat) | LD50 > 2000 mg/kg b.w |
| Inhalation (rat) | $LC_{50}$ (4 h) > 6.7 mg/l |
| Skin irritation (rabbit) | Not irritant |
| Eye irritation (rabbit) | Not irritant |
| Skin sensitization (guinea-pig) | Not a skin sensitizer (M&K) |
| **Chronic toxicity** | |
| Carcinogenicity | No suspected carcinogenic effects |
| Mutagenicity effects | No suspected mutagenic effects |

# **Ecological** information

| Aquatic organisms | |
|---|---|
| Fish | $LC_{50}$ (96 h) *Cyprinus carpio* > 100 mg/l<br>NOEL (96h) *Cyprinus carpio*> 100 mg/l |
| Daphnids | $EC_{50}$ (48 h) daphnia > 100 mg/l<br>NOEC 48 h) daphnia > 100 mg/l |
| Algae | $ECr_{50}$ and $EC_{b50}$ (72 h) *Scenedesmus subspicatus* > 100 mg/l<br>NOEC $_r$ and $_b$ (72 h) *Scenedesmus subspicatus*: 100 mg/l |
| Aquatic plants | Acute (7d) Lemna<br>$EC_{50}$ >100 mg/l |
| **Terrestrial organisms** | |
| Birds | LD50 bird > 2000 mg/kg bw (pNP)<br>LD50 bird = 1046 mg/kg bw (oNP)<br>LD50 bird = 2067 mg/kg bw (5-NG) |
| Bees | LD50 oral = 61.2 µg/bee (pNP)<br>LD50 oral = 123.2 µg/bee (oNP)<br>$LD_{50}$ oral = 131.6 µg/bee (5-NG)<br>LD50 contact = 111 µg/bee (pNP)<br>LD50 contact > 100 µg/bee (oNP)<br>$LD_{50}$ contact > 100 µg/bee (5-NG) |
| Earthworms | Earthworms (Eisenia fetida)<br>LC50 = 310 mg/kg soil<br>8 weeks NOEC = 37.0 mg/kg soil |
| **Persistence and degradability** | |
| Soil | DT50 in soil = 3.3 days (pNP)<br>DT50 in soil = 5.5 days (oNP)<br>DT50 in soil = 0.6 days (5-NG) |

Atonik has a very good profile regarding end user, consumer and environnement.