



# Biorational control options in store-product pest management with a special focus on breeding for pest-tolerance

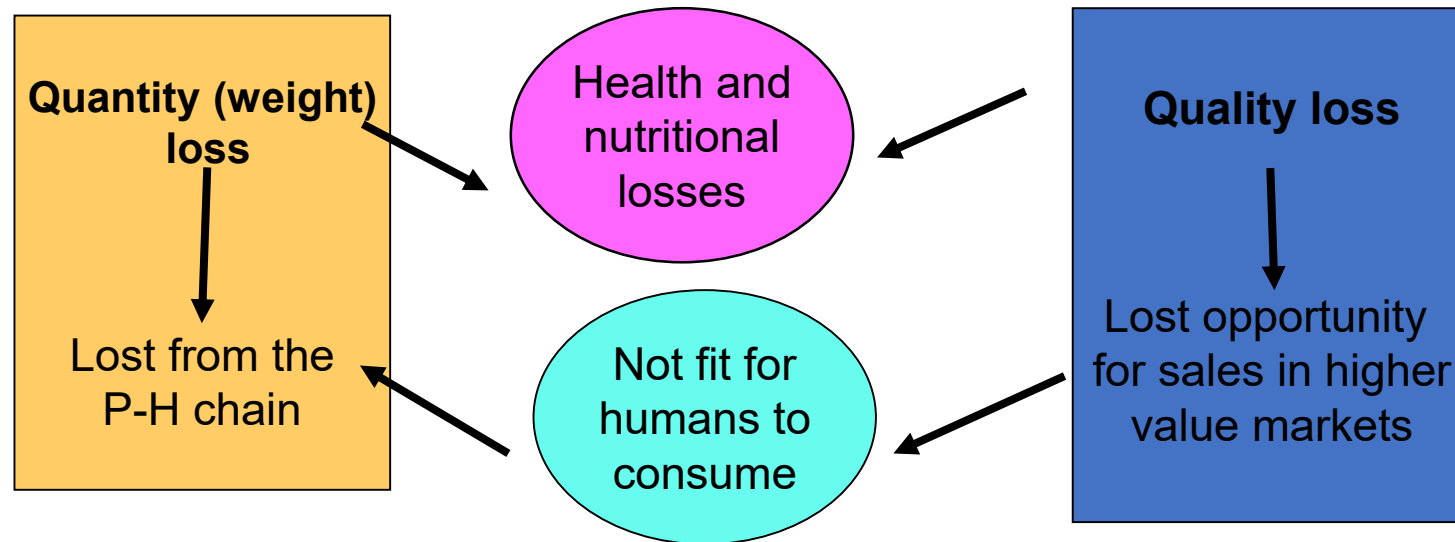
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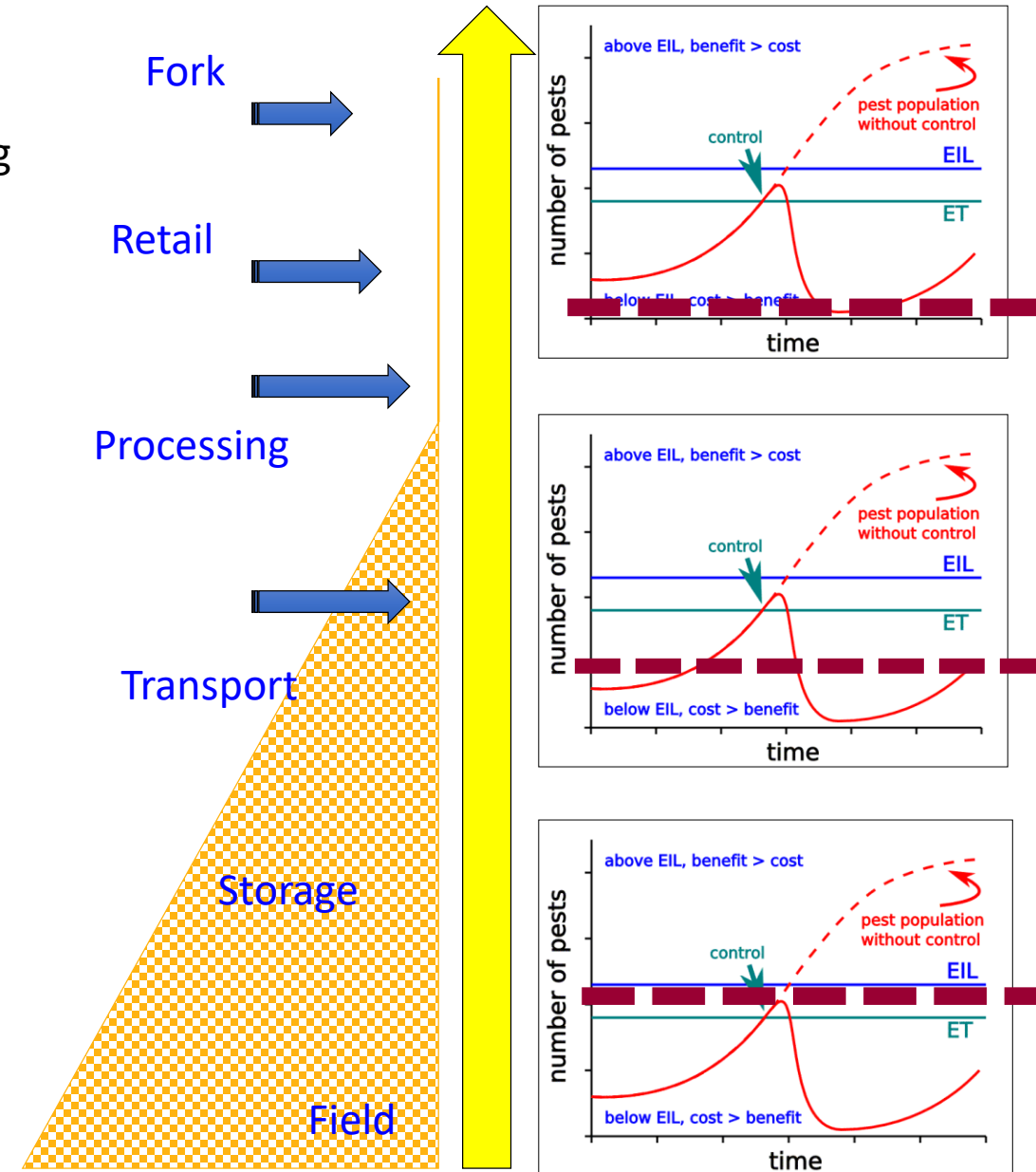


# BACKGROUND

- Sufficient amounts of stored grain (seeds) are considered a backbone of food security, particularly in periods when plant production fails.
- Stored product pests (SPPs) are a major cause of huge losses in commodities (raw and processed cereals, pulses, spices, dried fruit and nuts, feed, food...) during storage (10-90%).
- SPPs cause significant **QUANTITATIVE** and **QUALITATIVE** losses each year by feeding and development
- It results in reduced customer compliants, product rejection at the market, and cost associated with their management.



- In the last few decades, focus is on improving agricultural production, crop yields, **pre-harvest** implications to cope with globally increasing food demand and climate change.
- **PROTECTION of POST-HARVEST** as a vital aspect of food security are neglected.
- **PHLs** in durable agricultural commodities, especially pedigreed seeds (pre-basic and basic seed material), are **OVERLOOKED COMPARED WITH PRE-HARVEST PROTECTION** measures in practice (Fig).
- PHLs are neglected in the research sector as well, as less than **5% OF RESEARCH funding** has been allocated for research on PHL mitigation and storage technologies in previous decades



- Important aspect that contributes to PHLs is **climate change** as rising temperatures enhance outbreaks of SPPs.



## Effects of global warming on stored product insects

- Faster insect development
- More generations per year
- Tropical pest species migrate further up north
- More infestation in the field in cooler climates



Higher infestation pressure



Adler, C; Athanassiou, C; Carvalho, MO; Emekci, M; Gvozdenac, S; Hamel, D; Riudavets, J; Stejskal, V; Trdan, S; Trematerra, P 2022: Changes in the distribution and pest risk of stored product insects in Europe due to global warming ... JSPP 97, 101977

# WG Integrated Protection of Stored Products



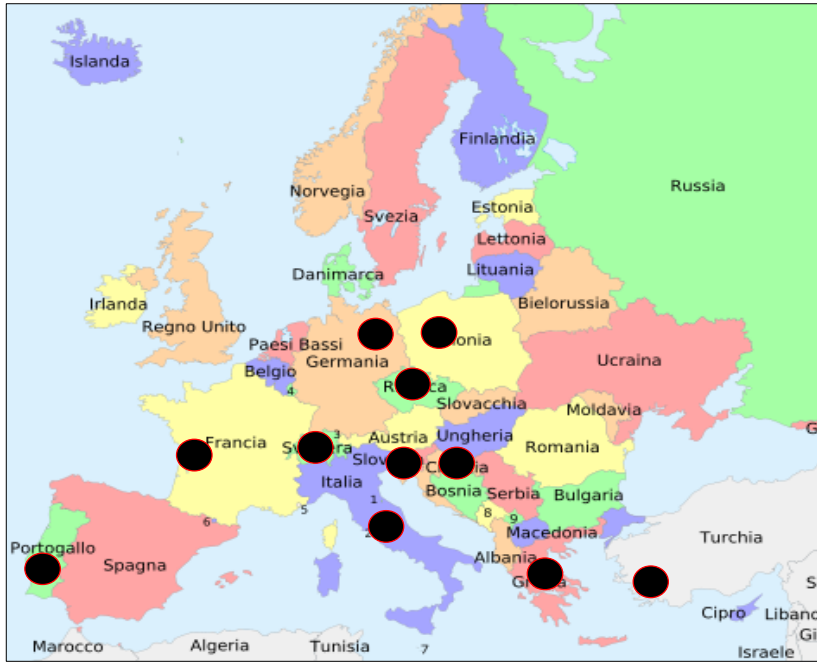
[www.iobc-wprs.org](http://www.iobc-wprs.org)

# Past



Marc Bardin  
Liaison

**Objective:** To promote the use of sustainable, environmentally safe, economically feasible and socially acceptable control-methods of pests and diseases of SPPs and food industries.



- **Last meeting 2022** (106 participants from 32 countries) (Local Organizer Dr. Nuria Agustí, IRTA)
- **Scientific topics:** Pest prevention during storage and transportation, Biology of stored product pests and diseases, and insects as food, Biological control, mating disruption and natural products, Chemical pest control, Modified atmospheres and physical pest control
- **Activities:** Publication of the Proceedings; Co-organization, co-operations and networking (COST, EUREKA, ERA NET, IWCSPP, ICE, CAF, ECE)

Meeting venues



# Future

- **Next meeting: Novi Sad, Serbia 16-20 September 2024**
  - *we expect a increase in the number of participants*
  - *Bulletin published before the meeting (new)*
- **Challenges/new developments?**
  - *Decrease in the number of experts in Universities / Research Centres*
  - *Increase interest in alternative control methods. However, limited commercial development of biological control*



## IPSP 2024



**14<sup>th</sup> Conference of the IOBC/WPRS  
Workig Group on the Integrated  
Protection of Stored Products**

**16<sup>th</sup> to 20<sup>th</sup> September 2024  
NOVI SAD, SERBIA**





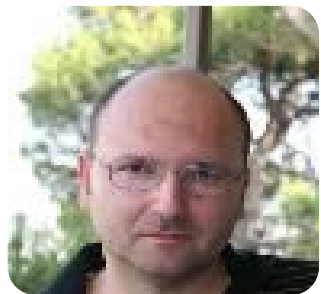
# CORE of SPP WG – An European initiative



**Jordi Riudavets, RTA, Spain**



**Cornel Adler, JKI, Germany**



**Christos Athanassiou,**  
University of Thessaloniki,  
Greece



**Pasquale Trematerra,**  
University of Molise, Italy



**Vaclav Stejskal,**  
Crop Research Institute, Czech  
Republic



**Sara Savoldelli,**  
University of Milan



**Stanislav TRDAN,** University of  
Ljubljana



**Sonja Gvozdenac, IFVCNS, Serbia**



**Maria Otilia Carvalho,** University of  
Lisbon, Portugal

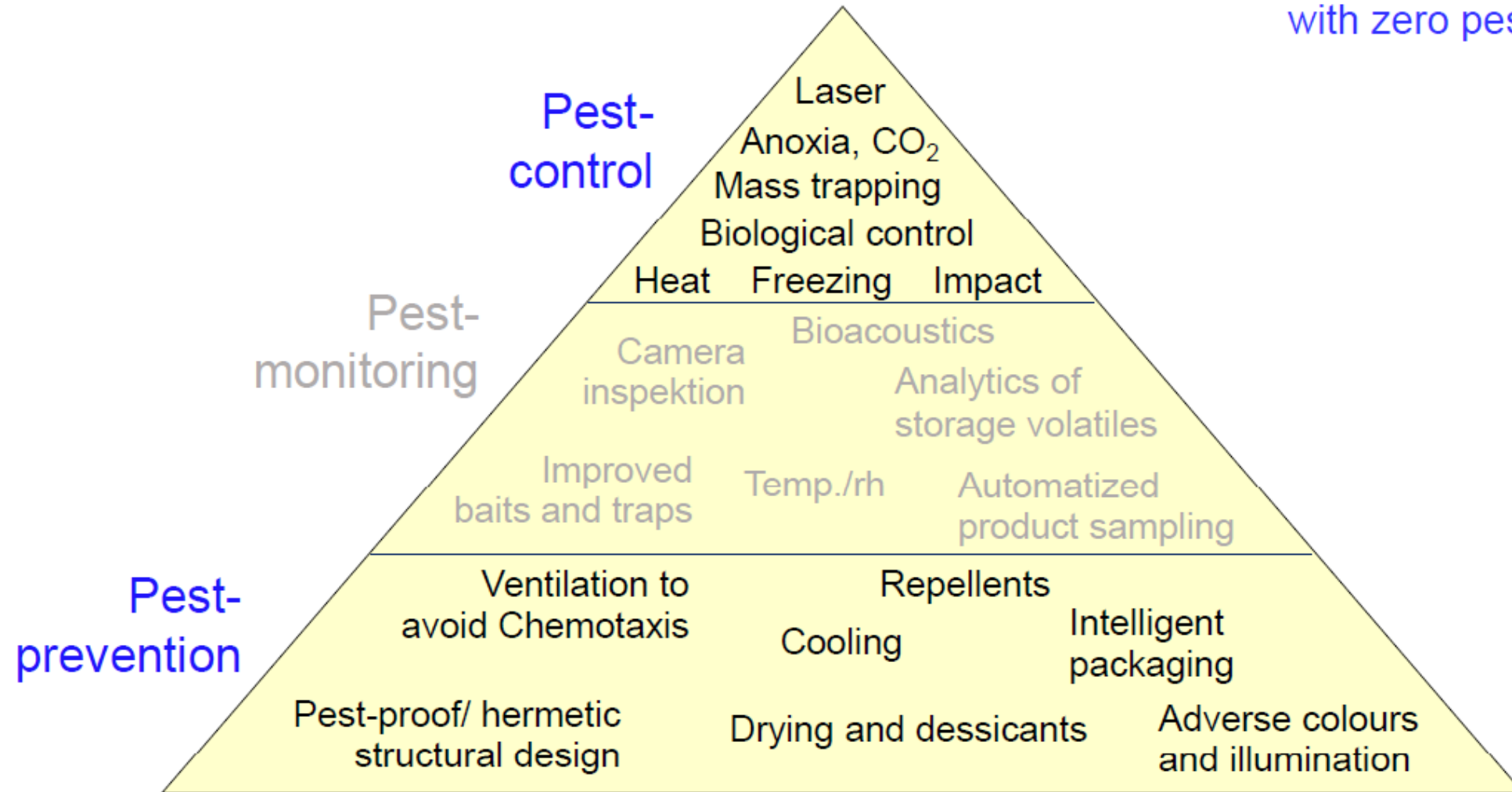
# Developing SPP control methods

- Up to recently, SPP management was globally related to the **use of chemical insecticides**
- **Results:** environmental pollution, the occurrence of pest resistance, residues in food and feed, negative impact on the environment, etc.
- **LEGISLATIVE CHANGES** at the European level and in 2009 - EU issued Directive 128/2009,
- Restriction or ban of large number of pesticidal compounds was initiated.
- **PLANT PROTECTION in front of a big challenge: to achieve satisfactory pest control with low chemical inputs.**
- A necessity to harmonize **SPP control with IPM principles** and use alternative methods for SPP control and **improve storage technology.**
- To move away from „calendar-based,, treatments and customize each treatment



# Elements of innovative technology for stored product protection

with zero pesticides



(Adler 2018)



# DIFFERENT APPROACHES IN SPP CONTROL

## Physical methods

Heating  
Cooling  
Controlled atmospheres  
    CO<sub>2</sub> fumigation  
    N<sub>2</sub> fumigation  
Hermetic storage -Modified atmospheres  
Vacuuming  
....

## Biotechnical methods

Pheromones  
    Mass-trapping  
    Attracticide (lure and kill)  
    Mating disruption  
    Autoconfusion  
  
Inert dusts  
  
.....

## Biological methods

Predators  
Parazitoids  
Pathogens  
Botanicals  
  
Cultivation method  
    Breeding for tolerance

# Heat treatment

[www.thermonox.de](http://www.thermonox.de)



Electromagnetic IR



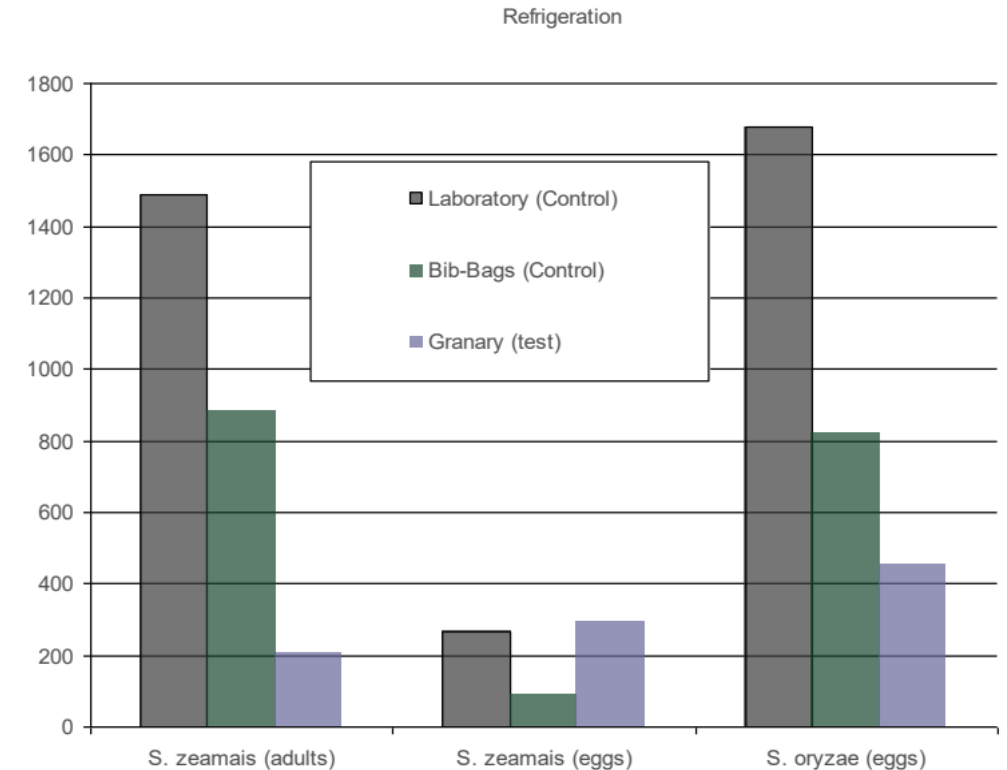
[www.biotech.at](http://www.biotech.at)

# Cooling

## Preventive strategies: the use of refrigeration for insect control and quality maintenance of paddy rice

- Testing reduction of *Sitophilus* spp activity
- The trials took place for 12 weeks during the hot season.
- Refrigeration was tested in a 40-ton silo
- Average temperature of  $17.8 \pm 0.4^{\circ}\text{C}$
- The results were compared under optimal pest development conditions (laboratory,  $28^{\circ}\text{C}$ ) and under real conditions (stored rice in big bags) in the rice factory ( $22^{\circ}\text{C}$ )
- Moisture content
  - Beginning 12%
  - End:
    - Treatment 13% moisture
    - Control 12% moisture

### Results



- F1 adult populations were **reduced by 71% to 77%** when compared to untreated controls.
- Refrigeration of the paddy at approximately  **$18^{\circ}\text{C}$**  allowed for storage for nearly three months and caused a delay in the development of rice weevils, eliminating the need for fumigation.
- Refrigeration provided an environmentally friendly and user-friendly treatment option during the warm months of the year



## CO<sub>2</sub> fumigation of sunflower and beans



### Biorational CO<sub>2</sub> fumigation of sunflower and common bean: insecticidal potential and effect on seed vitality and quality

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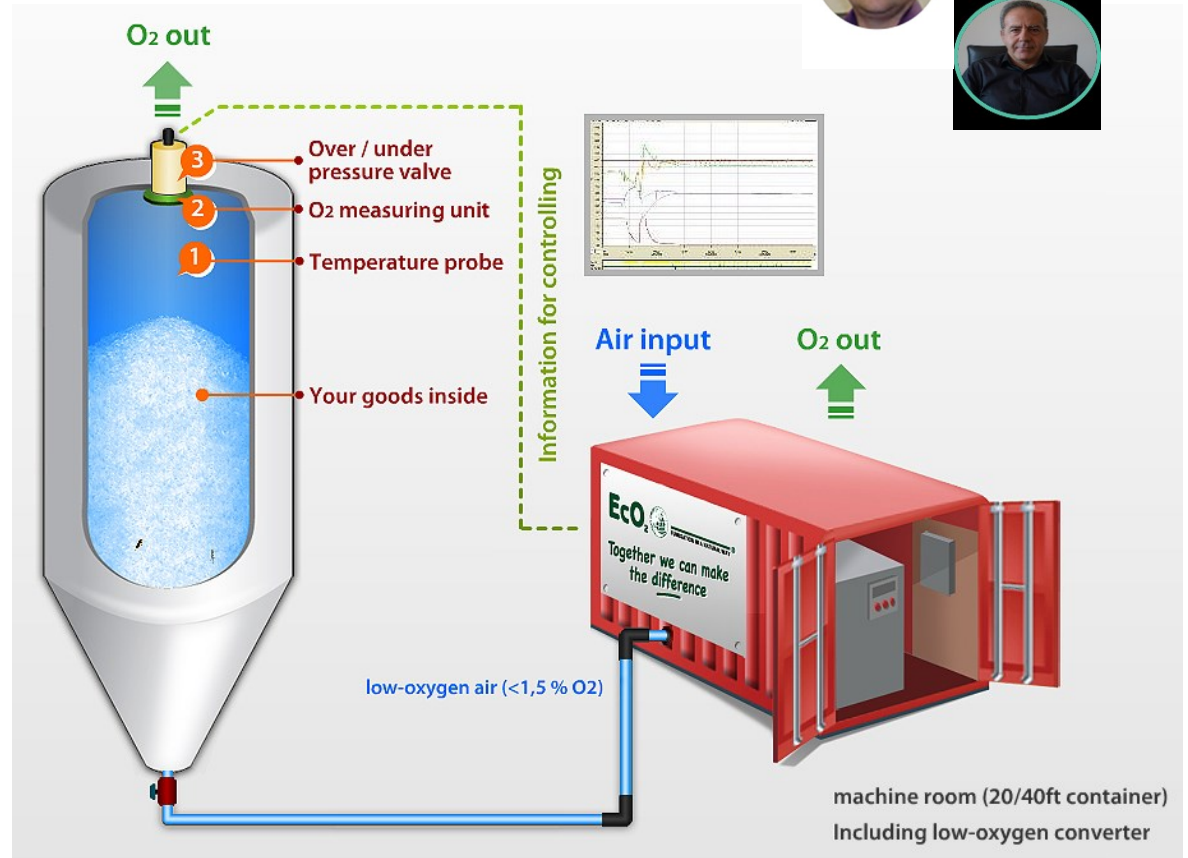
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**Abstract:** Store product pests often cause high qualitative and quantitative losses to seeds of agricultural plants during storage. Damages inflicted to a high category seed result in reduced germination that practically affects agricultural production. Therefore, it is important to control insect pests and mitigate losses in storages, but at the same time to preserve the germination potential of the seeds as well as their vitality and quality. Fumigation with CO<sub>2</sub> is a biorational method used for controlling store product pests in a variety of commodities. Its insecticidal potential is well documented, however the information about the effect on seeds is lacking. In this work, we assessed the efficacy of CO<sub>2</sub> fumigation and its effect on vitality (germination energy and germination) and quality (fatty acid composition) of sunflower and common bean seeds. CO<sub>2</sub> (62, 93 and 96 %), was applied to sunflower seeds artificially infested with *Plodia interpunctella* larvae and common bean infested with *Acanthoscelides obtectus* adults, in gas-tight bags. The lowest concentration (62 %) caused total mortality (100 %) of *P. interpunctella* larvae after 7 days of exposure. The two highest CO<sub>2</sub> concentrations caused relatively high mortality after two hours of exposure (81 and 86 %), while total mortality (100 %) was achieved after 24 h. The lowest concentration caused only 62.0 % mortality of *A. obtectus* after 24 h of exposure. In treatments with 93 and 96 % of CO<sub>2</sub> mortality was 88 and 93 % after 24 h exposure, respectively. Fumigation with CO<sub>2</sub>, irrespective of concentrations, showed no adverse effect on seed germination of sunflower (97.0 to 99.5 % in all treatments) or common bean (91.3-95.3 %), or on the percentage of detectable fatty acids in sunflower seeds. However,

- Effect on *A. obtectus* and *P. interpunctella* mortality
- CO<sub>2</sub> conc. 62, 93 and 96%
- Exposure of 7 days
- Average temperature of 25.0 ±1 °C
- The results: *A. obtectus* (93% mortality at 96% after 24h)  
*P. interpunctella* L5 (100% mortality - 62% for 7 days and at 93% for 24 h)
- No effect on seed GERMINATION

# Nitrogen (N<sub>2</sub>) fumigation



N generators in grains

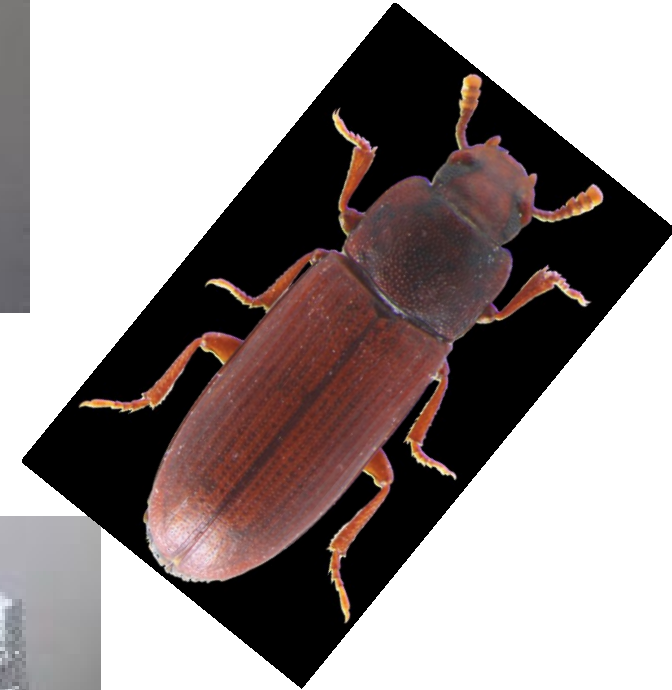


# Vacuum technology



CRI

CRI helped to develop and implement new technologies of controlled and vacuum atmospheres into practice



Aulicky R, Shah JA, Kolar V, Li Z, Stejskal V. Control of Stored Agro-Commodity Pests *Sitophilus granarius* and *Callosobruchus chinensis* by Nitrogen Hypoxic Atmospheres: Laboratory and Field Validations. *Agronomy*. 2022; 12(11):2748. <https://doi.org/10.3390/agronomy12112748>

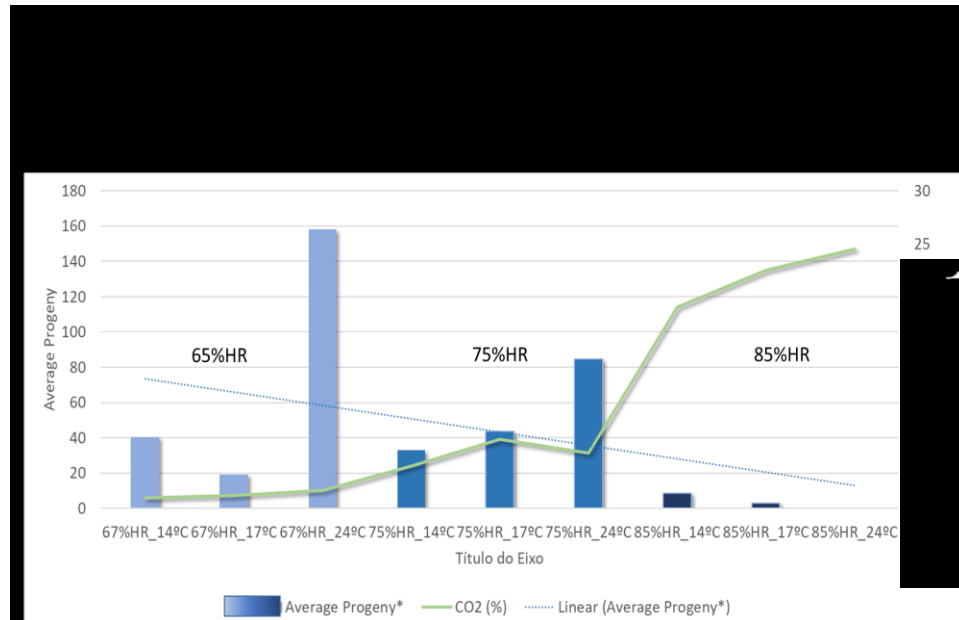
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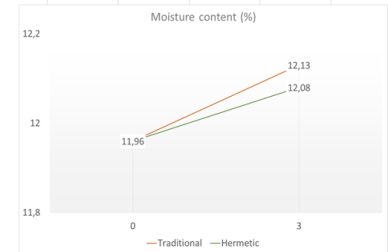
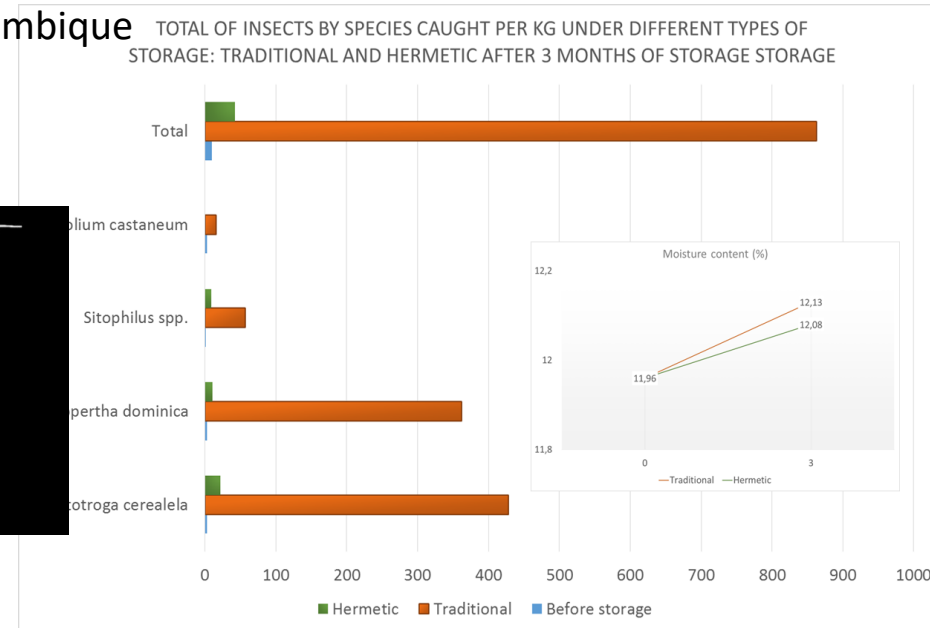
# Modified atmosphere

## The use of biogenerated atmospheres to control stored rice pests in Portugal and Mozambique

«**Biogenerated atmospheres** can be created in hermetically sealed storage systems. These atmospheres result from the respiration of living organisms and lead to oxygen-depleted and carbon dioxide-enriched interstitial atmospheres».

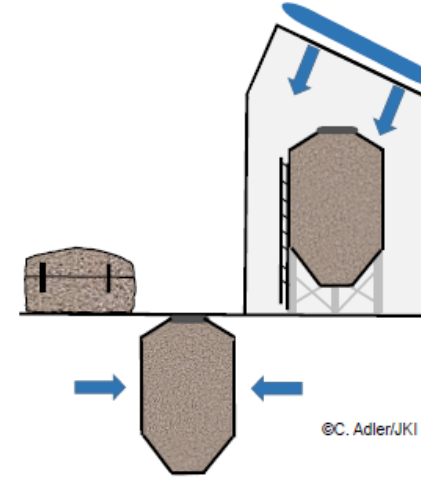


Mozambique TOTAL OF INSECTS BY SPECIES CAUGHT PER KG UNDER DIFFERENT TYPES OF STORAGE: TRADITIONAL AND HERMETIC AFTER 3 MONTHS OF STORAGE



**Portugal** [24°C under 85% RH] totally suppressed the insects, fungi didn't develop, rice flour maintained the rheological properties  
**Mozambique** reached 96% reduction of insect population Less 50% losses compared with traditional storage

## National project on field monitoring and hermetic storage: AVoiD



- Occurrence & distrib. of SP insects in storage/field
- Monitoring pest species and their dispersal
- Comparing rigid / flexible hermetic structures
- Above / underground hermetic grain storage (efficacy, economy, grain quality, sustainability)

First results may help to design EU-Horizon proposal

*Thank you!*

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# DIFFERENT APPROACHES IN SPP CONTROL



## Biotechnical methods

### **Pheromones**

- Mass-trapping
- Attracticide (lure and kill)
- Mating disruption
- Autoconfusion

### **Inert dusts**

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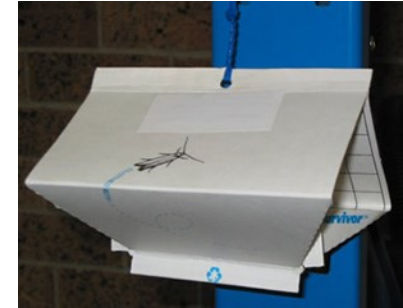
# Pheromones



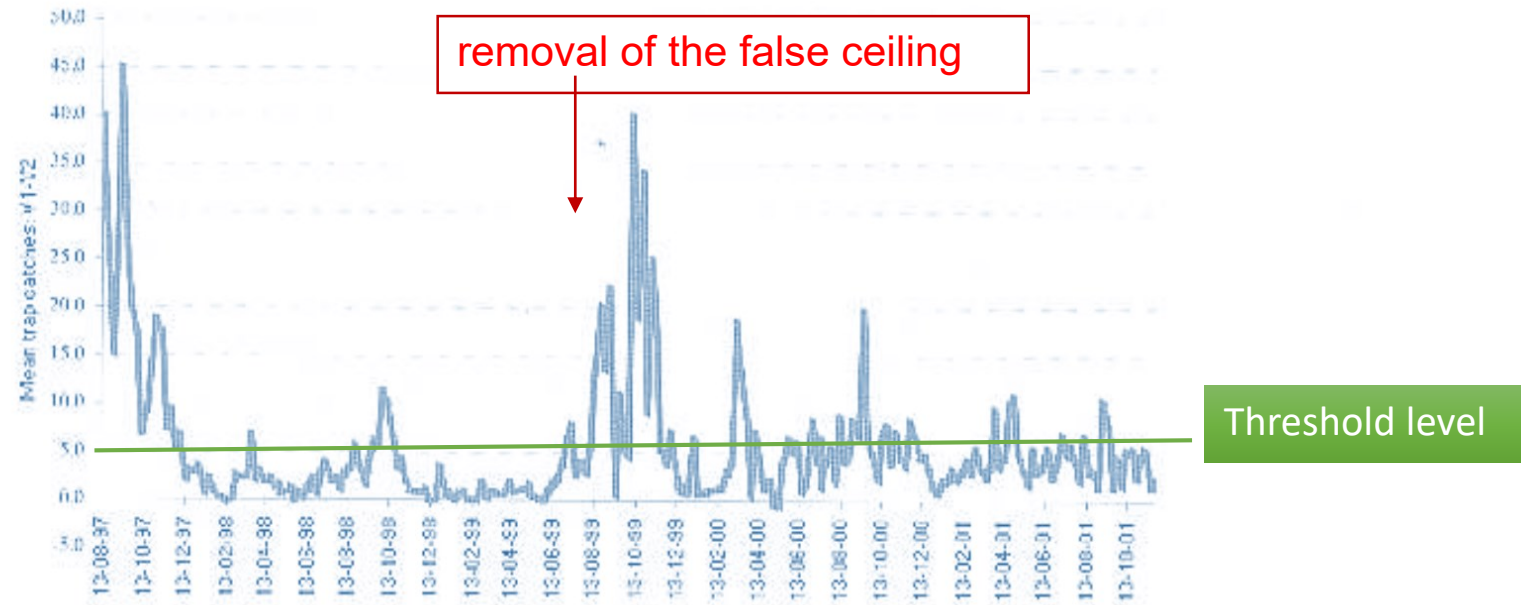
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DEL MOLISE

Considerable progress has been made in the use of pheromones for monitoring and control of SPPs by:

- Mass-trapping
- Attracticide (lure and kill)
- Mating disruption
- Autoconfusion



# Mass trapping of *Lasioderma serricorne* in a cigarette factory



In: Carvalho MO, Mexia A. 2003. The use of pheromone traps for mass trapping of *Lasioderma serricorne* in a cigarette factory in Portugal. In: Credland PF, Armitage DM, Bell CH, Cogan PM, Highley E. (Eds.), *Advances in Stored Product Protection. Proceed. 8th IWCSPP York, UK, CABI*, 222-229.

Mass trapping trials for *L. serricorne* conducted over 223 weeks in stored tobacco facilities (no chemical control)

Threshold is 5 insects/trap/week.

After **one year**, the number of insects caught **fell below this threshold**.

When the false ceiling was removed, it took an additional two years of mass trapping to reduce the *L. serricorne* population below the injury level.

- \* Mass trapping has a **medium and long-term effect** when dealing with high infestations.
- \* It is **most effective** when insect populations are **lower** because the sexual pheromone's power becomes more attractive and faces less competition from the male/female gender that releases the attractant.

The **aggregation pheromone is not as effective**



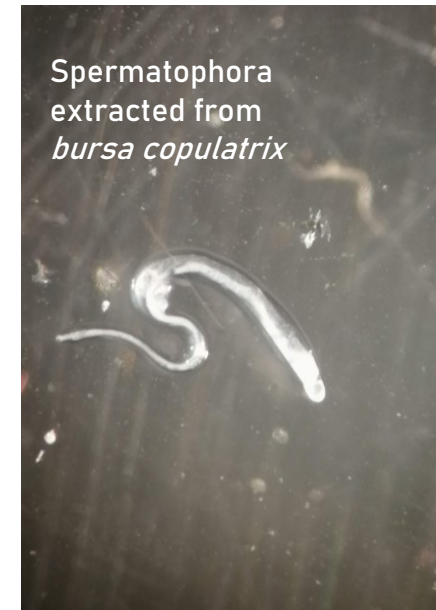
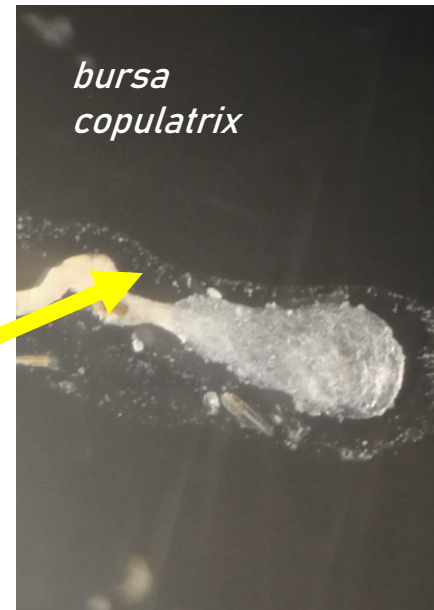
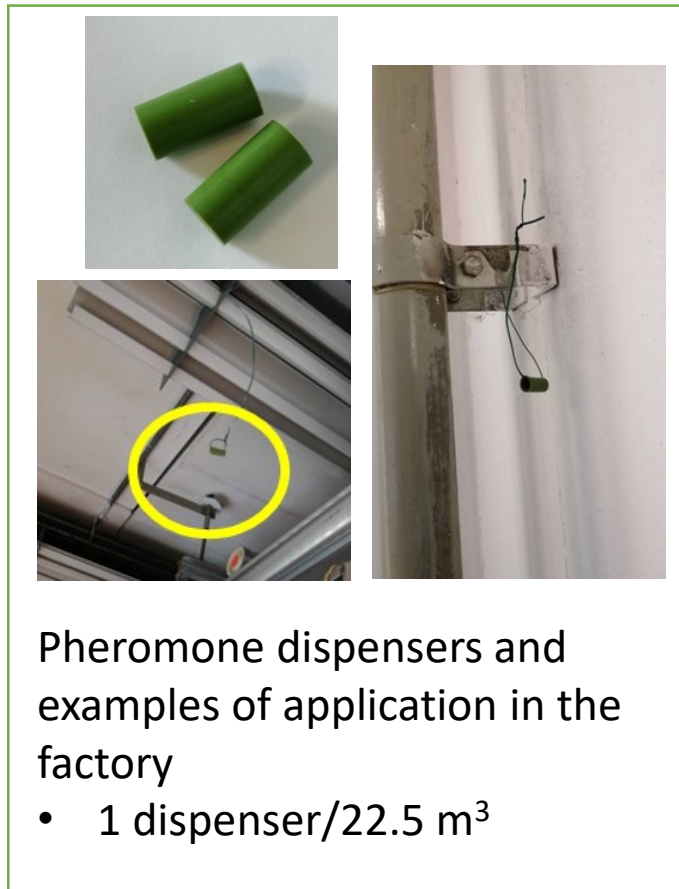
# Use of pheromone for managing *Nemapogon granellus* (European grain moth) infestations



- *N. granellus* is a polyphagous species: grain kernels, flours, dried fruit, dried mushrooms, various seeds, cured meat and cheeses

Test was made in a ham factory where *N. granellus* was present

- *N. granellus* adults were captured using water traps
- The female mating status was assessed (presence or absence of spermatophores, in the test area and control area)
- The reduction (%) of couplings was evaluated



# Use of pheromones reduced mated females

Journal of Stored Products Research 102 (2023) 102117

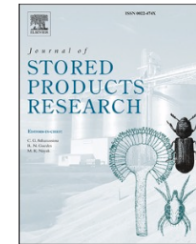


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## Journal of Stored Products Research

journal homepage: [www.elsevier.com/locate/jspr](http://www.elsevier.com/locate/jspr)



### Pheromone-mediated mating disruption of the European grain moth *Nemapogon granellus* in ham factories

Sara Savoldelli <sup>a,\*</sup>, Costanza Jucker <sup>a</sup>, Daniela Lupi <sup>a</sup>, Serena Malabusini <sup>a</sup>, Ezio Peri <sup>b</sup>, Salvatore Guarino <sup>c</sup>

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- Mated
- Unmated
- Unidentifiable

- Mated
- Unmated
- Unidentifiable

by reducing chemical treatments

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# Inert dusts

- **Inert dusts (ID)** are dry powders of different origins that are chemically unreactive in nature
- significant tool in IPM programs of **stored grain** providing insect control and preserving grain quality during storage.

## ➤ ADVANTAGES:

natural inert material,  
health safe  
effective insecticide,  
physical mode of action  
long persistence  
NOT leaving hazardous residues

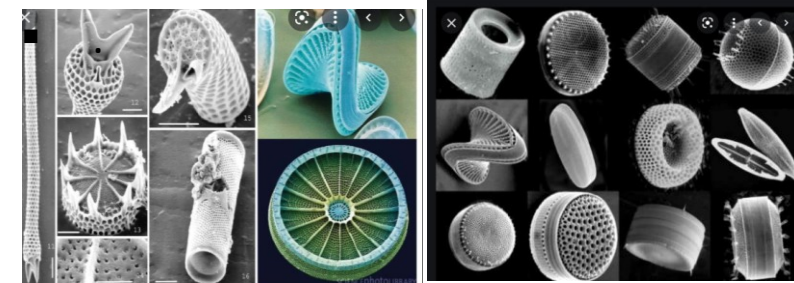
## ➤ DISADVANTAGES:

Increases hectolitar weight  
Reduces flowability of seeds

**Many DE dusts are commercially available and used in developed and developing countries for managing SPPs**



- DE contains 80 and 95% amorphous silicon dioxide
- composed of unicellular algae fossilized bodies called diatoms.



## EFFICACY OF DIATOMACEOUS EARTH IN CONTROLLING MAJOR STORE PRODUCT PESTS: *PLODIA INTERPUNCTELLA*, *TRIBOLIUM CONFUSUM* AND *ACANTHOSCLIDES OBTECTUS*

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### ABSTRACT

Diatomaceous earth (DE) is an inert dust formed from fossilized skeletal remains of diatoms. Insecticidal activity is a result of desiccation that occurs after DE particles destroy lipid layers of insect cuticle. Efficacy depends on chemical composition, particle size and geographic origin. This work assessed contact efficacy of DE originating from Kolubara open-pit mine (Serbia), in comparison to SilicoSec®, against *P. interpunctella*, *T. confusum* and *A. obtectus*. DEs were applied at rates: 5, 10, 15 and 20 mg<sup>-2</sup>. Mortality was recorded after 24, 48, 72 h and seven days. Significant mortality of *P. interpunctella* larvae was recorded after seven days of exposure at two higher rates of SilicoSec® (48.0 and 54%, respectively) and Kolubara DE (45.6 and 58.5%, respectively). Higher rates of SilicoSec® and Kolubara DE caused significant mortality of *T. confusum* after seven days (54.1, 84.3%, 49.2, 78.2%, respectively). High mortality of *A. obtectus* was recorded after 48 and 72 h in SilicoSec® (61.5, 82.1%, respectively) and in Kolubara DE (58.0, 78.5%, respectively) when applied at 20 mg<sup>-2</sup>.

**Key words:** alumina silicates, inert dusts, contact toxicity, storage pests

### INTRODUCTION

During storage, insects cause huge quality and quantity reduction of stored commodities and great economic losses (Puzzi, 2001). Therefore, the pest control is inevitable post-harvest measure that helps prevent damages and preserves the nutritional and commercial value of stored products. Concerns about rapid development of insecticide resistance, the environmental pollution and human health have intensified the search for alternative eco-friendly pest management strategies (Gvozdenc et al., 2018a). The use of materials like inert dusts, submicron and nanomaterials is one of the strategies that have been extensively tested as viable alternative to pesticides (Fields and Korunić, 2002) suitable for a long-term protection of stored products. Back in 1997, Golob first mentions the inert dusts as potent storage protectants in grain industry, with Diatomaceous earth (DE), being the most commonly used and evaluated (Golob, 1997).

OLUTUJI, O.F., OBIYA, T.I. AND ALADESANWA, R.D. 2010: Effect of particle size on insecticidal activity of dusts of *Eugenia aromatica* and *Piper guineense* against *Collosobruchus maculatus*. Nigerian Journal of Plant Protection **24**, 34-39.

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TOFEL, K.H., NUKEME, E.N., STAHLER, M. AND ADLER, C. 2015: Insecticidal efficacy of *Azadirachta indica* powders from sun- and shade-dried seeds against *Sitophilus zeamais* and *Collosobruchus maculatus*. Journal of Entomology and Zoology Studies **3**, 100-108.

### Effects of different inert dusts on *Sitophilus oryzae* and *Plodia interpunctella* during contact exposure

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DOI 10.5073/jka.2018.463.179

### Abstract

The use of natural inert dusts against storage insect pests is increasing recently, as an alternative to conventional insecticides. Laboratory study was carried out to evaluate the contact effect of three inert dusts, diatomaceous earth (DE), kaoline (KA) and vermiculite (VE), at rates 5, 7.5, 10, 15 and 20 gm<sup>-2</sup>, against adults of *Sitophilus oryzae* (L.) and larvae of *Plodia interpunctella* (Hubner). Insect mortality was evaluated 1, 2, 3 and 7 days after the exposure. Insect mortality varied depending on the species, concentrations and exposure periods. The DE and KA caused 86.7-98% mortality of *S. oryzae* after 2 days of exposure at the highest rates, while at 5 and 7.5 gm<sup>-2</sup>, 100% mortality was achieved only after 7 days. The highest rates of inert dusts caused 42-50% (DE) and 60-75% (KA) mortality of *P. interpunctella* larvae only after 7 days. The mortality of moths increased gradually with the concentration and 100% was achieved 3 days after the contact with DE and KA (10, 15 and 20 g m<sup>-2</sup>). However, inert dusts induced faster pupation of *P. interpunctella*, while adult emergence was reduced and adults had smaller body-sizes, compared to control. The VE caused relatively low mortalities (7-11% of *S. oryzae* adults and 5-8% of *P. interpunctella* larvae) at all tested rates during the entire experiment. Our results have shown good insecticidal effect of DE and KA against *S. oryzae* and *P. interpunctella* at 10, 15 and 20 gm<sup>-2</sup>. These products could therefore be used by small-scale farmers to protect stored grains against insect pest infestation.

**Key words:** Inert dusts, *Sitophilus oryzae*, *Plodia interpunctella*, contact exposure, diatomaceous earth

### Introduction

In recent years, the use of contact insecticides and fumigants for controlling storage pests is under increasing restriction due to the presence of residues in food and development of insect resistance (Collins, 2000; Kljajić and Perić, 2005). These shortcomings have stimulated the need for testing and evaluation of non-toxic methods that can replace conventional insecticides in stored grains (Arthur, 1996). Recently, physical control methods, like the use of inert dusts, have become prominent (Field and Korunić, 2002). These materials are classified into different groups depending on their composition and particle size and include materials such as diatomaceous earth, silicophosphate, rock phosphate, sand, kaolinite, clay etc. (Golob, 1997). There is a growing interest especially in desiccant or absorptive dusts, among which, diatomaceous earth is the most widely used in practice worldwide (Golob, 1997; Korunić, 1998a; Subramanyam and Roesli, 2000) and in commercial storages in the developed world. On the other hand, non-silica dusts and those composed of coarse grain silicates, such as kaoline and sand, have been used traditionally as grain protectants by small-



- Efficacy of inert dusts and plant powders against adults of *Sitophilus oryzae* under laboratory conditions



- Testing the insecticidal efficacy of wood ash and zeolites (LOCAL ORIGIN)



## DiatomiteThem

- Establishment of postharvest management protocols for commodities, applying Diatoms earth
- Assessment of economic efficiency for the proposed protocol
- Survey for assessing consumers' WTA and WTP for final foodstuff being treated with Diatoms earth



Co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (project code:T2EDK-3532)



## COMBINING AND INTEGRATING DIFFERENT MANAGEMENT TOOLS...

- heat combined with diatomaceous earth
- pheromones and pathogen
- diatomaceous earth and spinosad
- pheromones and food attractant oils
- push-pull strategies
- packaging + repellents....

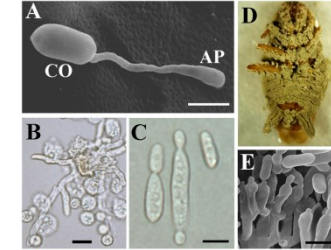
# Biological methods

Biological control has been attempted by using predators and parasitoids ([Titouhi et al., 2017](#)) or by applying plant essential oil treatments ([Jemâa, 2014](#); [Amzouar et al., 2016](#); [Titouhi et al., 2017](#)).

PREDATORS



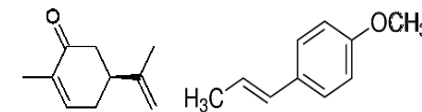
PATHOGENS



PARAZITOIDS



BOTANICALS



# Research objectives (II)

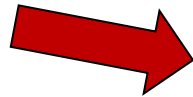
## Biological Control

Developing sustainable management systems compatible with the environment to allow the production of safe and high-quality food, improving the natural control of pests with the promotion of biodiversity and biological control

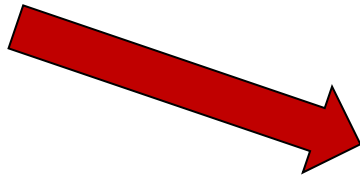
Field production



storage:  
Silos  
Warehouses



Food processing



Packaging



Distribution



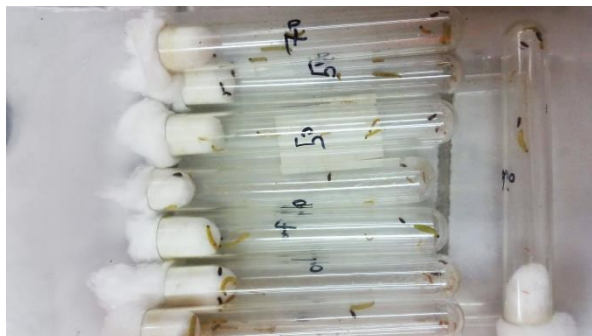
# BOTANICALS

## DIFFERENT RAW MATERIALS

- Invasive plants extracts
- Post-harvest residues extracts
- Essential oils
- Medicinal and aromatic plant extracts
- Minor-crops extracts

## BIOLOGICAL ACTIVITY OF BOTANICALS

- Fumigant
- Repellent
- Contact toxicity
- Contact-digestive



### Phytotoxic and Insecticidal Activity of Industrial Hemp (*Cannabis sativa* L.) Extracts against *Plodia interpunctella* Hübner—A Potential Sunflower Grain Protectant

Dejan Prvulović<sup>1\*</sup>, Sonja Gvozdenac<sup>2\*</sup>, Dragana Latković<sup>1</sup>, Marijana Peiš Tukuljac<sup>1</sup>, Vladimír Sikora<sup>2</sup>, Biljana Kiprovski<sup>3</sup>, Aleksandra Mišan<sup>3</sup>, Antonios Chrysargiris<sup>4</sup>, Nikolaos Tzortzakís<sup>4\*</sup> and Jelena Ovuka<sup>2</sup>

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**Abstract:** The biological activity (contact and contact-digestive toxicity, repellent and fumigant effects, effect on the insect's development and life cycle parameters) of industrial hemp (*Cannabis sativa* L.) ethanolic extract was assessed against *Plodia interpunctella*, the most destructive storage pest of sunflower. Additionally, the study aimed to examine the phytotoxic activity of the extract in order to assess its potential as a sunflower grain protectant. Phytotoxicity assessment was based on the effect on germination energy and seed germination and the activity of antioxidative enzymes, enzymes of the polyphenolic metabolism, and the intensity of lipid peroxidation in sunflower seedlings. The antioxidant capacity and content of phenolic compounds (total phenolics and t) also measured in seedlings. In the experiments, 70% ethanolic extract of dried industrial hemp (variety Helena) was applied at 0.5%, 1.0% and 2.0% concentration in control sunflower seeds. Ethanolic solution (70%) was the control. The hemp extract (1% medium repellence for *P. interpunctella* larvae (L<sub>3-4</sub>) while at 2% concentration it c mortality after 72 h. Moreover, the insect's development was prolonged and fecu reduced in hemp treatments. The extract did not exhibit fumigant activity. Germi germination of sunflower seeds were stimulated in treatment with 2% hemp e biochemical parameters of the seedlings were not significantly affected by the

**Keywords:** fiber hemp; *Cannabis sativa* L.; phytotoxicity; sunflower; *Plodia interpunctella*

#### 1. Introduction

Economic loss caused by insects is one of the main problems in the po

Received: 9 July 2022 | Accepted: 30 November 2022  
DOI: 10.1111/vea.13325

#### ORIGINAL ARTICLE

### Effect of four plant extracts on the mortality, population growth, and fluctuating asymmetry of *Sitophilus oryzae*

Jelena Ačanski<sup>1</sup> | Sonja Gvozdenac<sup>2</sup> | Marko Radenković<sup>3</sup> | Dejan Prvulović<sup>1</sup> | Snežana Tanasković<sup>3</sup> | Mladen Horvatić<sup>2</sup>

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**Correspondence:** Mladen Horvatić, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia. Email: mladen.horvatic@dbe.uns.ac.rs

**Funding Information:** H2020 Project: MATH4AGES, Grant/Award Number: 739570; Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant/Award Number: 451-03-47/2022-01/200022 and 451-03-47/2023-01/200558

**Abstract:** Management of storage pest insects relies heavily on chemical control. A need to develop more sustainable management practices. Here, we impact of 2% ethanolic plant extracts of *Ajuga reptans* L., *Ajuga reptans* Lamiaceae), *Urtica dioica* L. (Urticaceae), and *Cannabis sativa* L. (Cannabaceae) on mortality, population growth, and developmental stability (measured as fluctuating asymmetry (FA)) of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) stored product pest. FA refers to small, random deviations between the left and right sides of bilaterally symmetrical organisms; it increases in response to environmental stress, making FA a reliable measure of the impact of stress. FA was measured by means of geometric morphometrics. A method that allows for analyzing the whole landmark configuration rather than taking single measurements. Extracts of the mentioned plant to treat maize (*Zea mays* L., Poaceae) kernels on which experiment of the rice weevil were grown, and we assessed mortality after 24–77 growth after 30–90 days, and developmental stability after 90 days. *S. oryzae* showed that *S. oryzae* adults were most affected by *Ajuga reptans* extract especially *A. reptans*, significantly reduced population growth. In contact with *U. dioica* and *C. sativa* extract. None of the extracts significantly affected insect mortality. *C. sativa* concluded that *A. reptans* and *A. pyramidalis* are potential sources of compounds that may be further used for *S. oryzae* control. The results of variation in body shape asymmetry can be used as an indicator of population disturbance when insects are exposed to different types of stressors.



Contemporary Agriculture  
Serbian Journal of Agricultural Sciences  
Faculty of Agriculture, University of Novi Sad, Serbia  
[www.contagri.info](http://www.contagri.info)



Original scientific paper

UDC: 665.5  
DOI: 10.2478/contagri-2021-0020

### REPELLENT ACTIVITY OF *CYMOPOGON CITRATUS* ESSENTIAL OIL AGAINST FOUR MAJOR STORED PRODUCT PESTS: *PLODIA INTERPUNCTELLA*, *SITOPHILUS ORYZAE*, *ACANTHOSCELIDES OBTECTUS* AND *TRIBOLIUM CASTANEUM*

SONJA GVOZDENAC<sup>1\*</sup>, BILJANA KIPROVSKI<sup>2</sup>, MILICA AČIMOVIĆ<sup>3</sup>, JOVANA STANKOVIĆ JEREMIĆ<sup>3</sup>, MIRJANA CVETKOVIĆ<sup>3</sup>, VOJISLAVA BURSIC<sup>3</sup>, JELENA OVUKA<sup>1</sup>

<sup>1</sup>Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Maksimira Gorkog 30, Novi Sad, Serbia

<sup>2</sup>Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, Belgrade, Serbia

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\*Corresponding author: [sonja.gvozdenac@ifvcns.ns.ac.rs](mailto:sonja.gvozdenac@ifvcns.ns.ac.rs)

#### SUMMARY

The purpose of this study is to assess the repellent activity of lemongrass (*Cymbopogon citratus*) essential oil (EO), grown in Serbia under greenhouse conditions, against four prevalent stored product pests: *Plodia interpunctella* (larvae), *Sitophilus oryzae*, *Acanthoscelides obtectus* and *Tribolium castaneum* (adults). The lemongrass EO repellency was tested using filter paper in Petri dishes and a Y-tube olfactometer. According to the repellency index (RI), the lemongrass EO repellency was divided into 5 classes. Prior to biotesting, the chemical characterization of lemongrass EO was performed and the following main compounds were detected: myrcene (31.0%), geraniol (30.0%), and nerol (23.6%). The *C. citratus* EO considered was found to exhibit the Class III repellent activity against *P. interpunctella* larvae only at the highest concentration (namely 0.5%). This is the very first report on the *C. citratus* EO repellent activity against this pest. The lemongrass EO examined showed strong repellency (Class IV) against *S. oryzae* (0.2% and 0.5% of EO), *A. obtectus* (0.1% and 0.2%), and *T. castaneum* (0.05–0.1%). Moreover, higher lemongrass EO concentrations (0.5%) were found to exhibit extreme repellency (Class I) against *A. obtectus* and *T. castaneum*. The results obtained were confirmed in the bioassays performed, indicating the great potential of lemongrass EO as a bio-repellent when applied in higher concentrations to all the insects considered, regardless of the exposure period.

**Key words:** essential oil, lemongrass, biological activity, repellency, storage pests

**Abbreviations:** essential oil (EO); relative humidity (r.h.); repellency index (RI)

#### INTRODUCTION

Stored product pests have unique attributes that justify the need for specific control measures. Chemically synthesized insecticides have been used for decades to control harmful insects in storage facilities. However, these compounds have caused a number of negative effects such as environmental pollution, food and feed residues, ozone depletion, insecticide resistance, as well as human and animal health issues (Zettler & Arthur, 2000; Prado, 2003; Guelton, 2012; Foo & Hwang, 2010). According to the Directive 128/2000, the EU member states are obliged to

- **Insecticidal Potential of Plant Powders from Invasive Alien Plants against Rice Weevil under Laboratory Conditions**





CRI

CRI is conducting research on **protective packaging** of commodities and food: plant extracts as repellents



Fumigation of grain with essential oils in containers



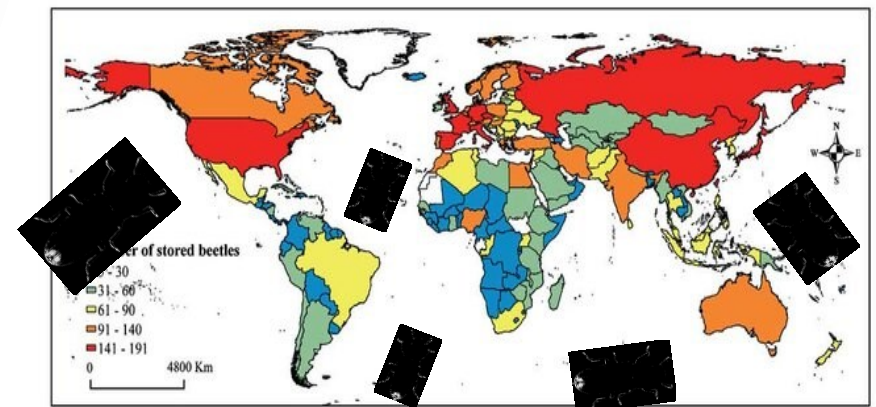
Treatment of primary, secondary, and tertiary packaging with repellent plant oils



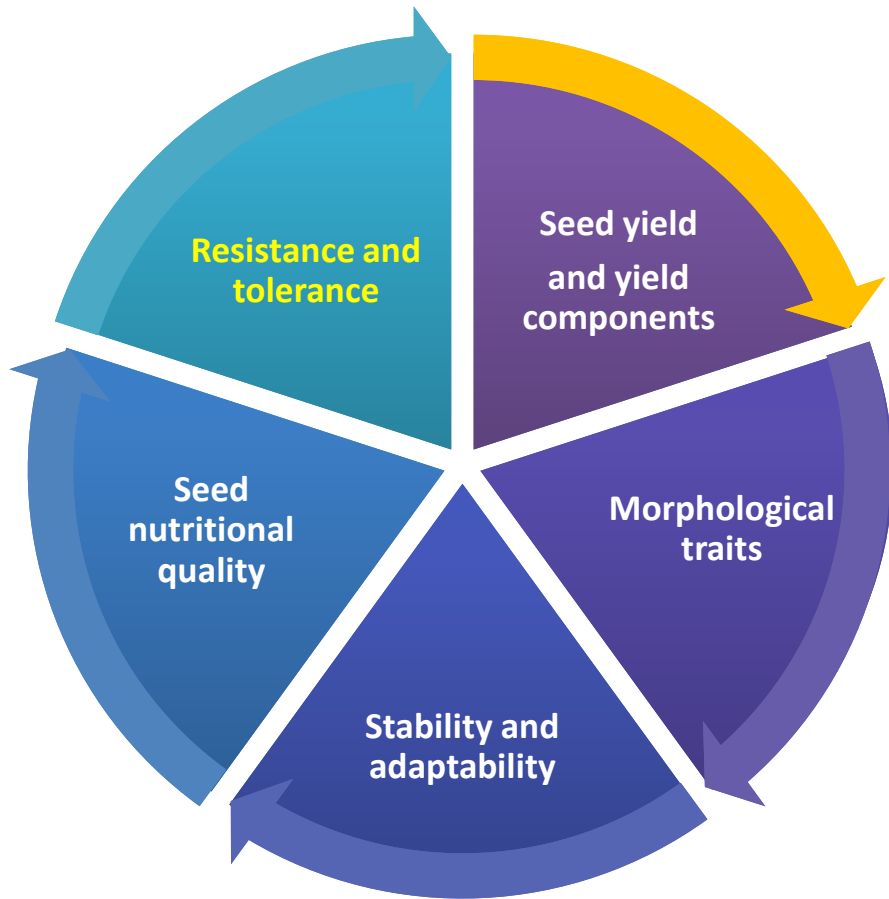
Vendl, T., Stejskal, V., Kadlec, J., & Aulicky, R. (2021). New approach for evaluating the repellent activity of essential oils against storage pests using a miniaturized model of stored-commodity packaging and a wooden transport pallet. *Industrial Crops and Products*, 172, 114024.

CAU (Beijing) and CRI elaborated maps of worldwide distribution of storage pests and risks of their future geographical spread

Qin, Y., Stejskal, V., Vendl, T., Zhang, Y., Li, T., Ullah, F., ... & Li, Z. (2023). Global analysis of the geographic distribution and establishment risk of stored Coleoptera species using a self-organizing map. *Entomologia Generalis*, 337-347.



# Breeding for tolerance



**Breeding objectives**



# Genetic variability

## WEAK POINTS OF CULTIVATED CROPS:

- NARROW GENETIC VARIABILITY
- DEFFICIENCY OF DESIRABLE GENES



## INCREASE OF GENETIC VARIABILITY:

- a) Using wild species
- b) Open pollinated varieties
- c) Mutations
- d) Target crossing



### Induced mutations

- changes in the genetic material caused by different chemical or physical means
- mutation breeding
- Mutagens: physical (X,  $\gamma$ , neutrons)
- chemical (ems, dms, sa)
- Mutational analysis:
  - Phenotype to gene (forward genetics): a wide array of characterised morphological mutants
  - Gene to phenotype (reverse genetics): until now no widely available resources

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www.icasilo-badine.rs

KONJANAN AGRICULTURAL RESEARCH, NO. 32, 2013  
Prist 1337 1222-4227, Opat 1337 247-5720

### NEW GENETIC VARIABILITY IN SUNFLOWER INBRED LINES CREATED BY MUTAGENESIS

Sandra Cvejić<sup>1</sup>, Sandra Jecić<sup>1</sup>, Milan Jeković<sup>1</sup>, Ivana Imirović<sup>1</sup>, Aleksandra Dmitrijević<sup>1</sup>,  
Dragana Milićević<sup>1</sup>, Slaven Prodanić<sup>2</sup>

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<sup>2</sup>Department of Plant Breeding and Genetics, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

### ABSTRACT

The successful use of plant breeding for improved desirable traits requires the existence of genetic variability for these traits. Induced mutations are often used to create new genetic variability within a plant species. The objective of this study was to provide new genetic variability that can be exploited for improvement of important agronomic traits in sunflower production. The seeds of 8 sunflower inbred lines (Crops, Novi Sad, Serbia) were irradiated with different doses of gamma radiation. The 360 generations (120000 plants) were selected for high oil content (OO) and high oil content per plant (OCP) in micro-plot tests in comparison with their respective parental line in hybrid combinations, as well as

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Genet Resour Crop Evol  
DOI 10.1007/s12042-015-0313-8

### RESEARCH ARTICLE

### The challenges of maintaining a collection of wild sunflower (*Helianthus*) species

Jovanka Atagić<sup>1</sup> · Sreten Terzić<sup>1</sup>

Received: 9 April 2015 / Accepted: 1 September 2015  
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www.springerlink.com



### Genetic variability for concentrations of essential elements in tubers and leaves of Jerusalem artichoke (*Helianthus tuberosus* L.)

Sreten Terzić<sup>1,2\*</sup>, Jovanka Atagić<sup>2</sup>, Ivana Maksimović<sup>3</sup>, Tijana Zaremski<sup>4</sup>, Miroslav Žonić<sup>5</sup>

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### ARTICLE INFO

Article history:  
Received 13 August 2015  
Revised 10 March 2016  
Accepted 20 December 2015  
Available online 22 January 2016

Genetic variability  
Crops  
Sunflower  
Jerusalem artichoke  
Tubers

### ABSTRACT

Due to the diversification of essential elements in the Jerusalem artichoke (*Helianthus tuberosus* L.) on cultivated agricultural land, a collection of wild sunflower (*Helianthus*) species was established to ensure the genetic variability of essential elements in tubers and leaves and to assess the potential ability of accessions to breeding programs aimed at improving the quality of cultivated Jerusalem artichoke tubers and leaves. Concentrations of selected essential elements in tubers and leaves were significantly different, not all the analyzed essential elements in leaves, Na, Ca, Mg, K, Mn, Zn and Cu. Ca was the most abundant element in tubers. The level of all elements, which varied significantly, was also different in leaves. The leaves contained higher concentrations of P, Fe, K and Cu. In the Jerusalem artichoke leaves, there was an adequate amount of analyzed essential elements for use as an animal feed.

It was found that tubers contain an adequate amount of major and microelements when used as food, and the elements content was similar to or greater than in the other root crops. An accession with the lowest Na/Ca ratio was also an accession from Montenegro with ratio of 1/3 which is still higher than the proposed ratio for ratio level of up to 1/3.

By monitoring of wild sunflower accessions through molecular analysis, it was shown that a group of accessions from Montenegro has particularly the best mineral composition for further work. It is possible solution to increase leafy crop and animal element ratios in leaves, and tubers could be the source of them resources for cattle feed, but further studies are required before a conclusion on this matter can be made.

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### 1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) belongs to a polymeric grass genus *Helianthus* L. It is one of the most cultivated species on the territory of today's United States of America together with the cultivated sunflower *H. annuus* var. *maximilianii* (DC.) Gilg. (Silson, 1955).

Interest in Jerusalem artichoke as a cultivated plant started through health, and often used in the periods of food shortages (Kojic and Nottingham, 2008). Today, it is mainly seen as an ornamental crop (Li et al., 2010), but it is also used for production of sugar and other special tissues for animal feed, where the dietary role of starch is emphasized (Chikmagal et al., 2010). When compared to other cultivated plants, Jerusalem artichoke is highly efficient on energy production, and those who cross (Loring et al., 1992). The source of high adaptability, Jerusalem artichoke can be grown well almost in all environments, it grows well and yields high, however in each condition it has lower yields and quality lowers fertility of the soil which then has to be prepared for the next crop (Congrove et al., 2006).

Tubers represent the basic reproductive organs of Jerusalem artichoke and they develop by rhizome thickening during the accumulation of nutrients (Mies and Switzer, 1976). They contain approximately 88% of water, 15% carbohydrates and 1–2% proteins.

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doi:10.1016/j.sci.2015.09.014

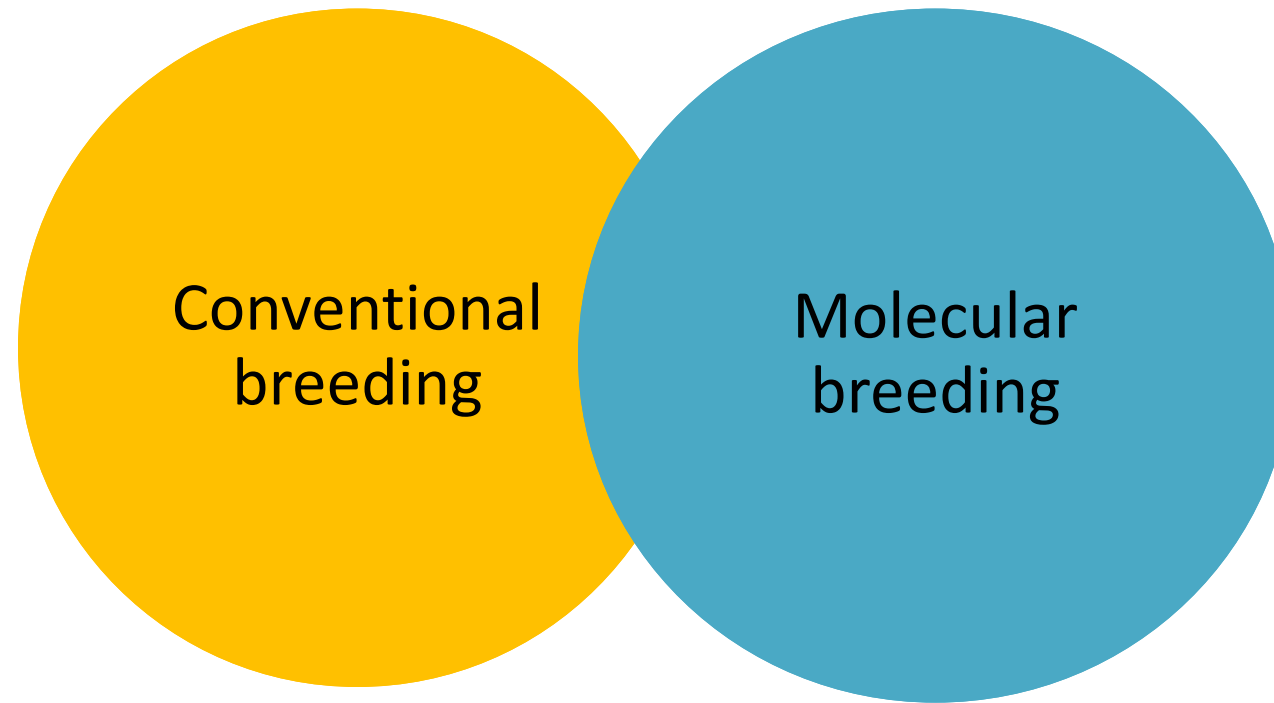
In crop improvement is well...

emphasis on using pea and a Hagar and Hodgson (2007) is a steady increase in the need of adult crops with an addition of the Consolidative Group of Research (CGCR), Wheat, rice and sunflower have been

sorted by the number of traits. Wild sunflowers have a as a source of genes for diabetes, herbicide, abiotic tolerance of oil quality and in cytoplasmic male sterility genes, important in F<sub>1</sub> hybrid source of genes for crop sunflower species significantly variability of sunflower at mid March 2011.

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## Breeding methods



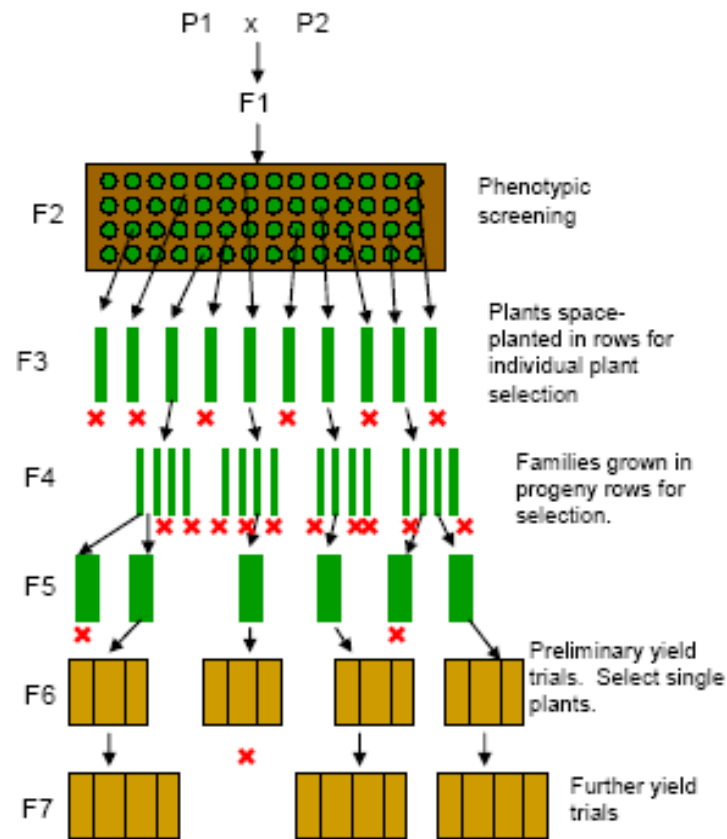
- Mass selection
- Individual selection
  - Bulk selection
  - Pedigree method
  - Single seed descend
  - Back-cross method

- Marker assisted selection (MAS)
- Genomic selection (GS)
- Genome editing
- Genetic modification –Transgenic plants

# MAS – identification of genotypes with specific gene

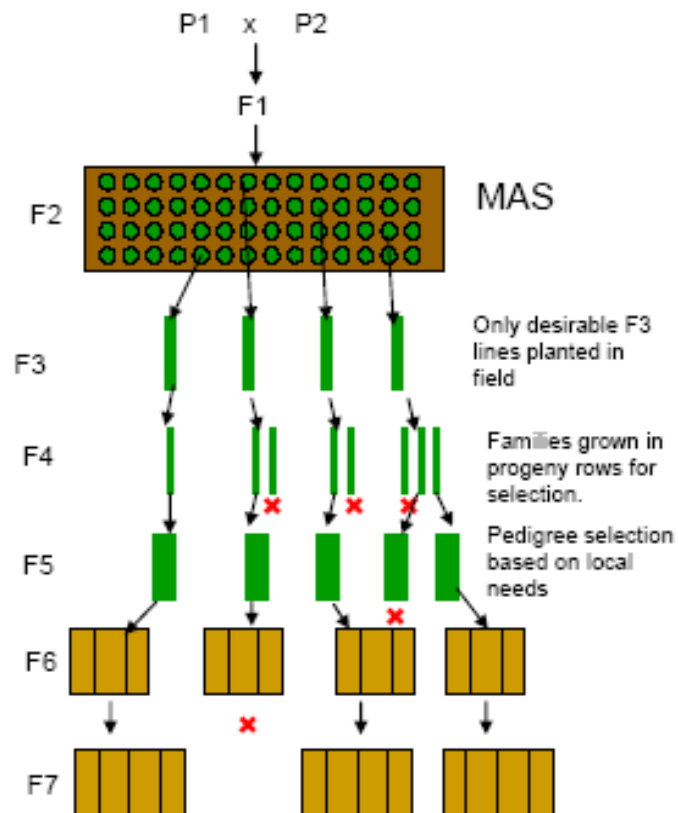


## PEDIGREE METHOD



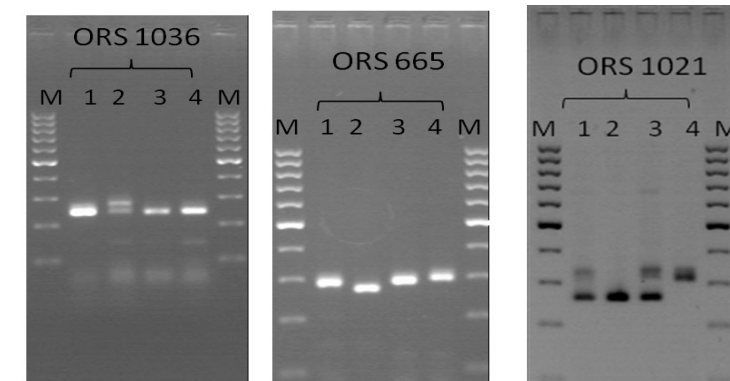
F8 – F12 Multi-location testing, licensing, seed increase and cultivar release

## EARLY GENERATION SELECTION MARKER ASSISTED SELECTION



F8 – F12 Multi-location testing, licensing, seed increase and cultivar release

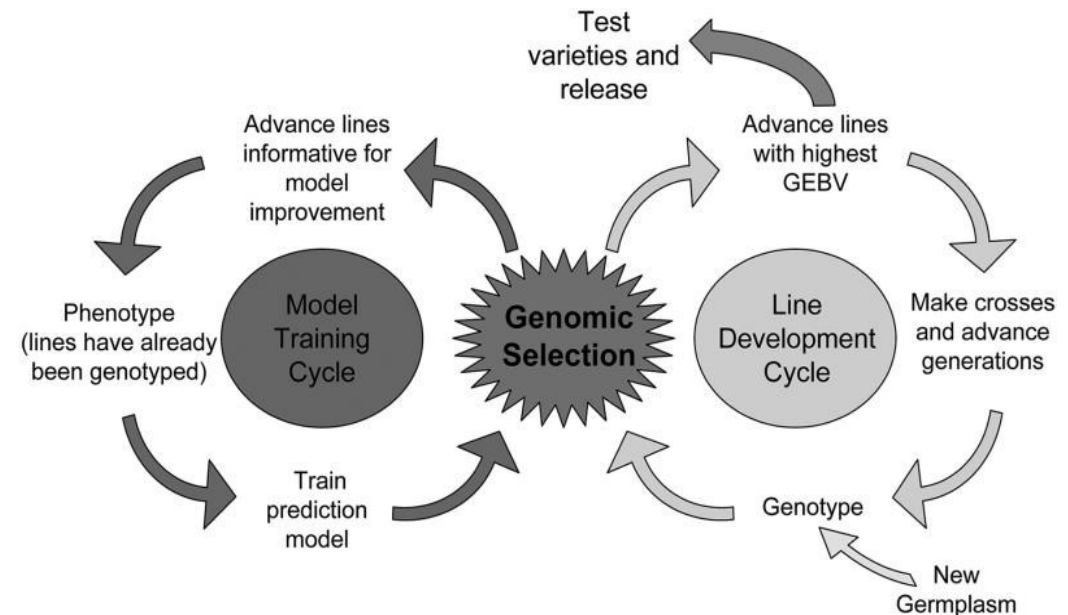
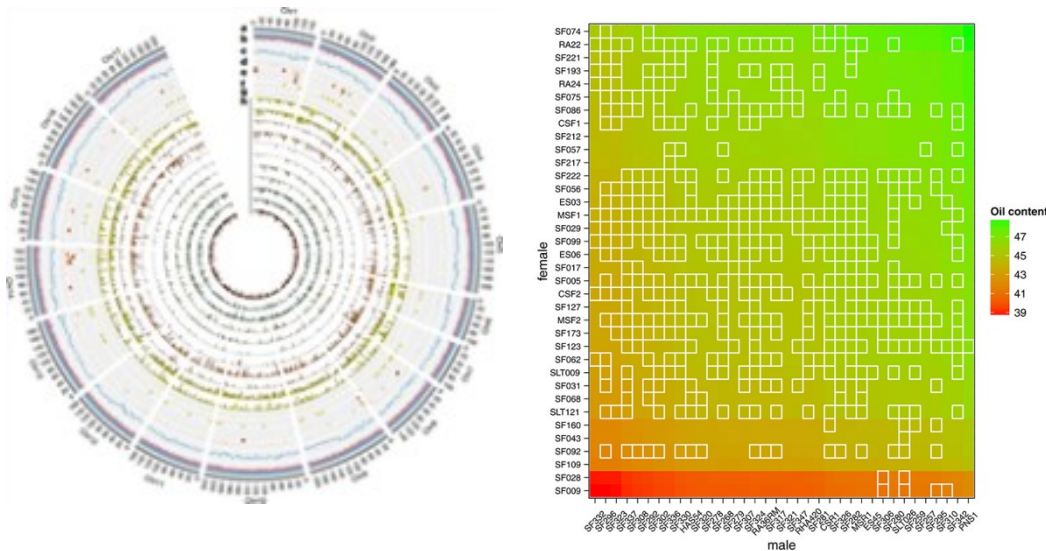
- Mapping a new gene that controls tolerance
- Reliable only for monogenic traits
- SSR, SNP
- AFLP, RFLP, CAPS markers



# Genomic selection



- GS = Genome-wide prediction
- Method for improvement efficacy of selection of plant quantitative traits
- GS uses genotypic and phenotypic data from the population to calculate quantitative value of each individual as a parent for future breeding cycles – it is called genome-estimated **breeding value**
- **Suitable for polygenic traits** (Quantitative Trait Loci -QTL markers)
- MAIZE genom sequenced
- BEAN genom sequenced
- SUNFLOWER genom sequenced (used to predict hybrid performances (Reif et al. 2013), oil content in hybrids (Mangin et al. 2017) and *Sclerotinia* tolerance (Livaja et al. 2016)).

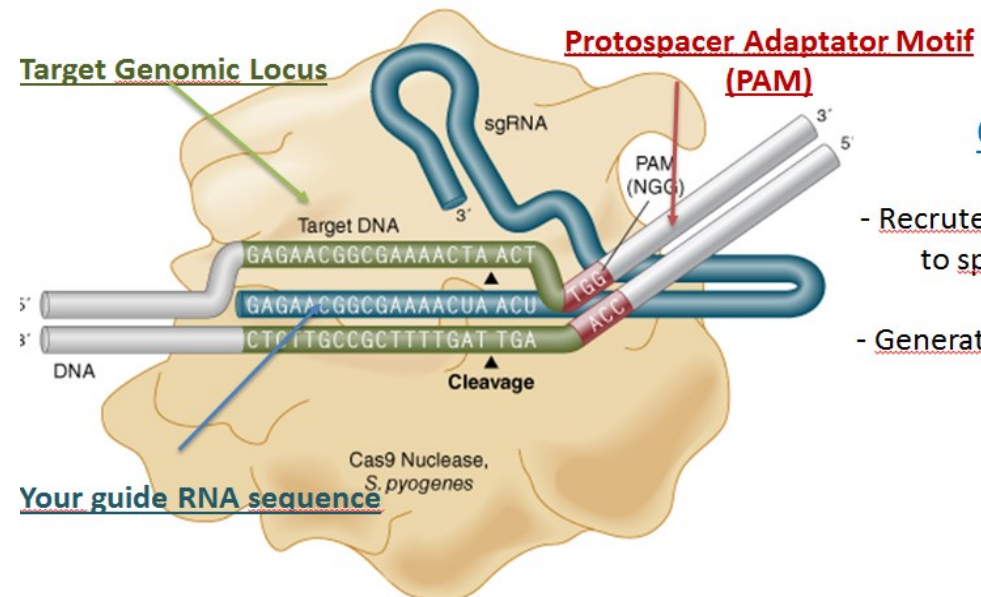




# Genome editing



- In 2016 Nobel prize (in Medical sciences)
- Group of laboratory techniques that change DNA structure (insert gene of interest in DNA structure) at a molecular level
- CRISPR-CAS9 – new genomic tool for locating a region of interest
- In EU the application and even research was arguable



E. Charpentier/J. Doudna

# Breeding for SPP tolerance



**Antixenosis** (a non-preference) - a plant characteristic poses a chemical or biophysical barrier that deters or repels the insect without causing it harm.



**Antibiosis** is a process of biological interaction between two or more organisms that is detrimental to at least one of them.

It reduces the survival and reproduction of the insect or prolongs the time between generations; reduces the rate of initial insect population.

**Tolerance** - plants have the ability to grow and yield even when attacked by the pest (or a resistance in which a plant is able to withstand or recover from damage caused by insect pest equal to that damaging a plant without resistance characters (susceptible).



# Breeding for SPP tolerance in practice – Maize case

## Genetic bases

- Maize weevil resistance has been reported to be under the control of ADDITIVE and NON-ADDITIVE GENE ACTION
- The involvement of GENES WITH ADDITIVE EFFECTS for resistance suggests that grain weevil resistance is controlled by **minor polygenes** and CAN BE improved by selection
- differences of 16–49% for maize weevil resistance between divergently selected maize populations, demonstrating the possibility of improving grain weevil resistance by selection
- Promising inbred lines and experimental crosses identified can be effectively utilized in the resistance breeding programme



# Breeding for SPP tolerance in practice – Bean case

## Genetic bases

- Detected markers that are common for bean resistance to weevils.
- The researchers found **three regions of bean DNA** important for weevil resistance.
- Three QTL for resistance to *A. obtectus* were identified on chromosomes Pv04 and on Pv06
- One of the QTL on Pv04, named as AO4.1<sup>SA</sup>, was previously reported as the arcelin, phytohemagglutinin and  $\alpha$ -amylase, (APA) resistance locus



## Key steps prior to breeding proces



- Identify and define traits responsible for seed tolerance to SPPs
- Identify which genes are responsible for certain trait
- See if a trait is regulated by one or more gene (monogenic or poligenic trait)
- Choose/ Define tolerant lines/genotypes as candidates for breeding
- Make genome-wide prediction if possible



## Suitability of different maize hybrids for development of *Plodia interpunctella* (Hübner)

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**Abstract:** *Plodia interpunctella* (Hübner), the Indian meal moth (IMM), is a major lepidopteran storage pest worldwide. IMM larvae can be found in high abundance in stored maize and cause huge losses in seed germination and seed viability. This work aimed to assess the susceptibility of six maize hybrids (NS 6140, NS 640, NS 1090, NS 444 - dent type, NS 620k - pop-com type, Red-aleurone maize - maize with an altered aleuron color) to IMM attack and suitability for pest's development. Standard laboratory diet (SLD) was used as a positive control. Under laboratory conditions, the following IMM life history parameters were monitored: larval mortality, mean developmental duration (egg to adult),

susceptibility was determined by larval mortality and fecundity. Larval mortality of IMM larvae was 11.2–19.5%. The highest and the lowest on NS 620k (115.8 eggs), on NS 6140 and NS 640 (8.5) on SLD (115.8 eggs), on

### Suitability of Poaceae seeds for *Plodia interpunctella* development

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DOI 10.5073/jka.2018.463.036

### Abstract

One of the most important pests of stored grains is *Plodia interpunctella* (Hübner), whose larvae feed primarily on germinal part of the kernels, causing a reduction of seed germination and seed viability. This is detrimental for seeds of high category. However, seeds of different species within the same taxonomic family have different morphology (thickness of seed-coat, presence or absence of palea, palea loose or firmly attached to the seed etc.), which affects the susceptibility of seeds to *P. interpunctella* attack. The hypothesis was that seed hardness and the absence of palea could also significantly influence the life history of this pest. We assessed the suitability of different seeds from family Poaceae (maize, wheat, barley, oats, ray, forage sorghum (variety), forage sorghum (hybrid), Sudan grass and millet) for *P. interpunctella* development and seeds susceptibility to pest attack (expressed in Susceptibility index –SI). The following parameters were monitored: larval mortality, adult emergence, mean developmental duration (from egg to adult) and female fecundity. Observations were carried out weekly, for 49 days. Data were statistically analyzed using Duncan's multiple range Test. The highest larval mortality, the lowest number of emerged moths and the lowest fecundity were recorded on millet, Sudan grass



## Life history of *Plodia interpunctella* Hübner on sunflower seeds: Effects of seed qualitative traits and the initial seed damage

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### ARTICLE INFO

Article history:  
Received 13 July 2018  
Received in revised form  
29 August 2018  
Accepted 29 August 2018

### Keywords:

*Plodia interpunctella*  
Development  
Fecundity  
Sunflower types  
Seed damage

### ABSTRACT

Sunflower seeds are regularly infested by *Plodia interpunctella* d damaged seeds, in practice it can infest undamaged seeds as well the sunflower seed type (oil, protein for human consumption) during post-harvest processing (dehulled kernels, 10, 20, 30% oil on development of *P. interpunctella* (larval mortality, larval development and emergence and fecundity). Biochemical analysis of seeds content of phenols in the seed and hull and tocopherols in the oxidative activity was the highest in the seed, kernel and hull shortest development (39.5 days) and the highest fecundity (91 longest development (42.1 days) and the lowest fecundity (68.1) bird feed. The highest mortality of larvae was on the undamaged and human consumption (21.3% and 14.0%, respectively). The damage affected larval mortality, developmental duration at duration and the number of emerged adults were dependent on component analysis detected strong positive correlation between tocopherol content on the undamaged seeds while protein was the amount of tannins, proteins and oil content in the seed. The bird feed were the least suitable for the development of this most suitable.

tus L.) is the most important oil crop of the four major oil crops in the world (Balalić et al., 2012). According to

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FAO estimates, it is cultivated in more than 70 countries (FAOSTAT, 2016). In 2016, production in 2016 was 34.0 million compared to 2014, followed by 2015 and 2016 (FAOSTAT, 2016). It is cultivated: the oil type for the non-oil type (protein content

## Are protease (trypsin) inhibitors responsible for suitability of different legumes for *Acanthoscelides obtectus* development?

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## Suitability of three different legumes for *Acanthoscelides obtectus* development and population growth

### Pogodnost tri različite vrste leguminoza za razviće i rast populacije *Acanthoscelides obtectus*

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Received: December 20, 2022; accepted: May 19, 2023

### ABSTRACT

Legumes are a rich source of valuable nutrients thus represent important component in human and animal nutrition. The most important and often a limiting factor in legume production is the presence of seed pests, such as the bean weevil *Acanthoscelides obtectus* (Say, 1831). This work tested the suitability of three different legume species (common bean, faba bean and grass pea), the species with a growing interest in the human diet, for the development of the bean weevil, aiming to provide a reliable forecast of its population growth. After four months, bean weevils consumed the highest percentage of the common bean kernels (70.79%), followed by the grass pea (53.13%), and faba bean (0.42%). Growth were significantly affected by the tested legume species. After each month of the observation, the bean weevil emerged specimens after each month of the observation, the bean weevil emerged specimens also on the grass pea. The lowest number of emerged adults, in all indicating its low preference and suitability for the weevil's development. The as the highest on the common bean, followed by grass pea, and it fitted best to led the prediction of the population growth of the bean weevil for each legume



- The suitability of wheat grain and grain of three less common cereals as hosts for the rice weevil

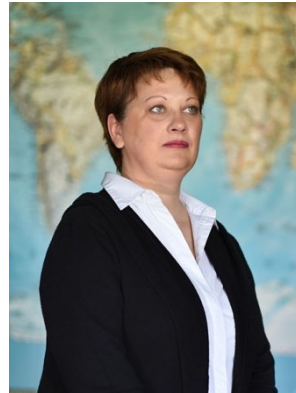
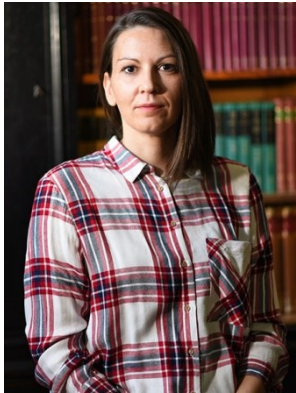




## CONCEPT

- 10 crop species (maize, wheat, barley, oat, rye, millet, sunflower, bean, faba bean, grass pea)
- Over 30 genotypes of each crop species
- Detailed biochemical analysis (macromolecule content, micromolecule content, antioxidant stress components...)
- Phenotyping (morphological and anatomical characteristics)
- Genetic analysis
- Metabolomic and proteomic analysis (polysaccharides and protease inhibitors, protein composition)
- Machine learning and artificial networks - TOLERANT IDEOTYPE
- Insects (*S. oryzae*, *P. interpunctella*, *A. obtectus*, *T. castaneum*, *R. dominica*)
- TRAITS: life cycle parameters (MDD, fecundity, progeny production...), feeding indices, mortality, population growth models etc.





# Future Prospects in Breeding for SPP tolerance



## On improved phenotypic strategies and analytics:

- sequencing and genotyping costs continue to drop, phenotyping has been highlighted as one of the most costly and major limitations to the precise mapping of traits in plant breeding
- Need to develop and utilize next-generation phenotyping platforms that facilitate the accurate, low-cost, and timely acquisition of phenotypic data for enhancing the data quality
- The application of modern analytics is vital to maximizing phenotypic data outputs. For instance, the analysis of data using mixed models would enhance the heritability values for genomic analyses, ML and AN.

## Advances in plant bio-chemistry:

- the use of plant biochemistry for revealing the basis of pest resistance to SPPs
- low-cost analysis throughput platforms such as near-infrared spectroscopy (NIRS) are required, since wet chemistry platforms like liquid chromatography mass spectrometry (LC-MS) are still costly for chemical resistance profiling.

## Advances in genomics:

- It is currently more important than ever that more molecular markers, particularly SNPs, are identified since this would fasten genomic breeding for SPP resistance
- The completion of the whole-genome sequencing of cultivated crops in the near future will further enable the maximum exploitation of the genomics-assisted selection

# Take away message!



- Host plant resistance is a very promising approach for SPP management.
- Attempts to develop resistant cultivars have yielded in case of maize (USA) and legumes (Asia and Africa)
- The underlying genetic and bio-chemical basis for resistance has been understood to some extent and molecular markers such as SNPs have been developed, though still not being fully utilized in breeding.
- More effort will be needed by breeders to develop additional markers for mapping SPP resistance to facilitate marker-assisted selection and genomic selection.
- Moreover, the global advancements in seed phenomics, proteomics, biochemistry and metabolomics will eventually bring forth cutting-edge approaches for seed improvement that will eventually lead to accelerated genetic gains in breeding for resistance to SPPs.

**Thus breeding of resistant cultivars is the most appropriate approach to achieve durable and efficient levels of resistance that meet the requirements of the agri-food sector and promote sustainable agriculture.**



**Thank you for your attention**

