



ASSOCIATION
TUNISIENNE DE BIOTECHNOLOGIE

PRODUCTION OF BIOPESTICIDES BY *BACILLUS THURINGIENSIS* ISOLATED FROM LEBANESE AND TUNISIAN SOIL

– «STUDY OF ECOTOXICOLOGICAL EFFECTS AND EFFICACY TESTS OF BIOPESTICIDES BASED ON *BACILLUS THURINGIENSIS* “LIP” AND “BLB1” AGAINST *PHYLLOCNISTIS CITRELLA* AND *PRAYS CITRI*» –

Presented by Rayan NASSEREDDINE
European Project: IPM-4-Citrus (No 734921)

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4/18/2023
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Plan

ATBiotech – IDB2022 – 20th December



- General introduction
- Objectives
- Experimental methodology
- Results and discussion
- Conclusion et Perspectives

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General introduction

IPM-4-CITRUS

From Research From Lab ...to Market ...to Field

Integrated

Pest

Management

6 years (2017-2023)

HORIZON 2020 FUNDED
Marie Skłodowska Curie Action
Research & Innovation Staff
Exchange

11 partners 6 countries 1 GOAL

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General introduction

WP1: Management / Coordination

- Meetings & Conferences
- Round Tables
- Workshops Open days
- Informal Education

WP2: Proof of concept

- INSAT 100-1000L
- FS-SJ
- FS-USJ
- MEDIS 1-10L
- CBS BT Microbiology
- INSAT/TWB Lab scale

WP3: Activity optimisation (highlighted in red)

- IPT / ENTOMO BT impact
- BBU Field assays
- MEDIS Pilot scale
- JKI Lab scale

WP4: Economic Maturation


- MA¹
- WSU
- TA²
- RA³
- BIPCA
- MEDIS
- DEP⁴

WP5: Communication, Networking & Outreach Activities (all partners)

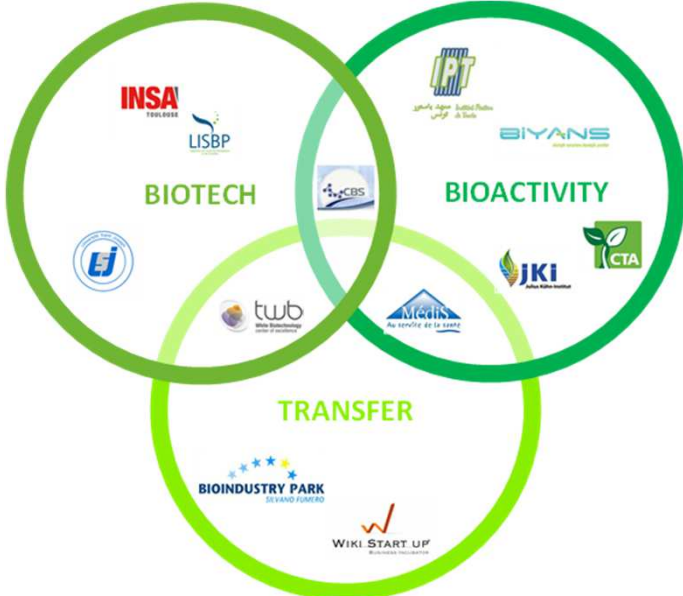
WP6: Dissemination & Exploitation


1. MA = Market Assessment / 2. TA = Technology Assessment / 3. RA = Regulatory Assessment / 4. DEP = Definition of Exploitation Paths

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General introduction






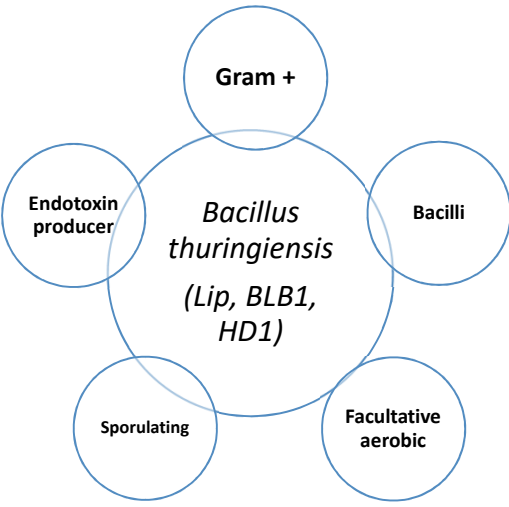
WP3

Conducting researches to optimize the efficiency and safety of the products

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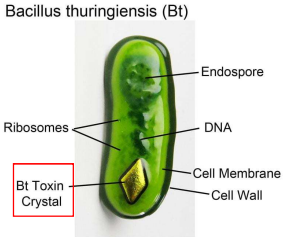


General introduction



δ-Endotoxin

- ✓ Insecticidal activity
- ✓ Non toxicological effects for plants, animals or humans



Bacillus thuringiensis (Bt) life cycle and endotoxins production (www.u.osu.edu)

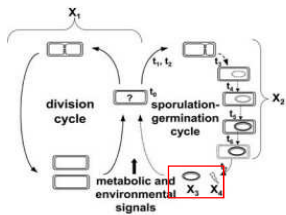


Fig. 1. Different physiological states in a spore-forming bacterium. X_1 : the biomass with ability of division; X_2 : biomass in sporulation process; X_3 : free spore; X_4 : sporangial debris; t_0 : normal growth; t_1 : genetic system division; t_2 : asymmetric septation; t_3 : engulfment; t_4 : cortex synthesis; t_5 : coat synthesis; t_6 : lysis of mother cell; t_7 : spore liberation.

(Sarrafzadeh et al., 2005).

General introduction



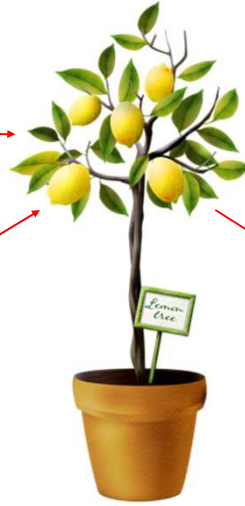
Leaves

The leaf miner : *Phyllocnistis citrella*



Fruits

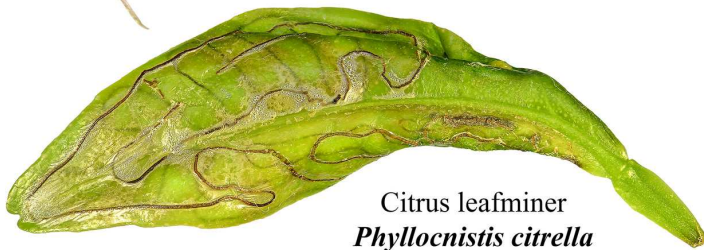
The citrus flower moth : *Prays Citri*



General introduction



← 2nd instar



Citrus leafminer
Phyllocnistis citrella

Objectives



USJ ----> BIYANS (Çukurova university)

1. Efficacy tests:

- Evaluate the effect of **delta-endotoxins (δ -endotoxin)** on target pests (*Phyllocnistis citrella*, *Prays citri*) in fields.
- Determine the effect of BLB1 and LIP formulation in controlling citrus diseases (*Phyllocnistis citrella*, *Prays citri*) on lab scale.

2. Spore's persistency test:

- Test of spore residual through the field assay - Colony forming unit (CFU)



Field assays



Experimental methodology

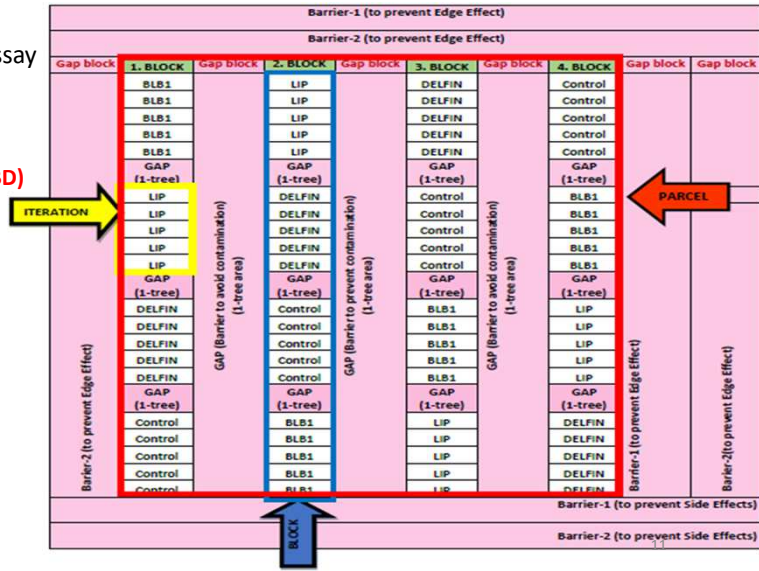
1. Efficacy experiments

- *Prays Citri* and *Phyllocnistis citrella* field assay

FIELD SPRAY DESIGN

The Randomized Complete Block Design (RCBD)

- Two barriers to prevent edge effects
- Four treated blocks
- Vertical gap block between treated blocks
- Four iteration for each treatment
- One horizontal tree gap between each iteration



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Experimental methodology

1. Efficacy experiments

- *Prays Citri* and *Phyllocnistis citrella* field assay

Biopesticide	Protein concentration (g/L)	formulated product (g)	Water volume (L)*	Total formulated product (g)	Total volume (L)**
BLB1 mix_2022	1.050	9.375	15	37.5	60
LIP mix_2022	0.833	11.250	15	45	60
DELFIN WG	0.607	15	15	60	60
Water	0	0	15	0	60

(*): Volume prepared for one iteration: Each iteration contains 5 trees, and each tree needs 3 L; that means we need 15 liters per iteration.

(**): Total volume prepared per biopesticide: We have four iterations per biopesticide; that means that we need 60 liters in total.

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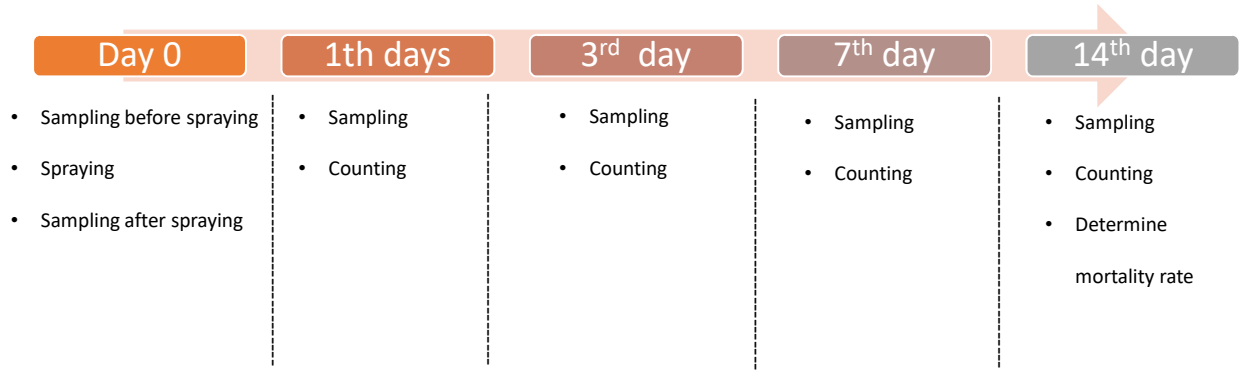
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Experimental methodology

1. Efficacy experiments

- *Prays Citri* and *Phyllocnistis citrella* field assay

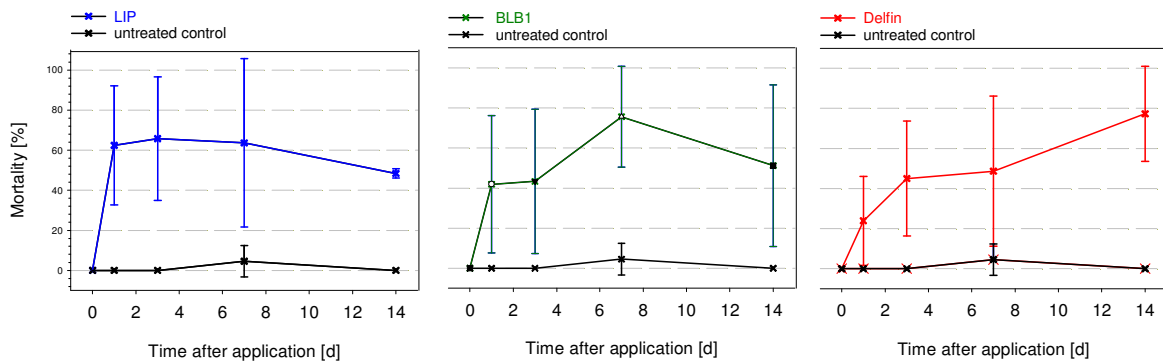


R&D1: Field assay

1. Efficacy experiments

- *Prays Citri* and *Phyllocnistis citrella* field assay

Prays citri

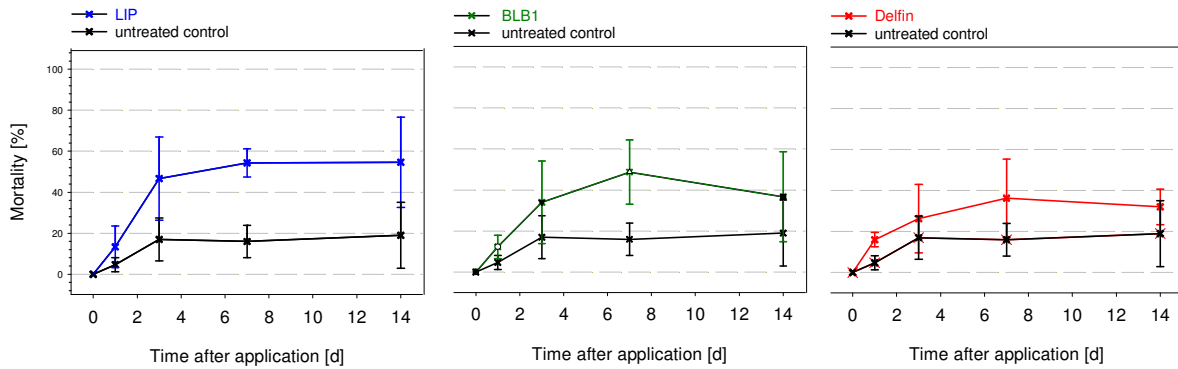


R&D1: Field assay

1. Efficacy experiments

- *Prays Citri* and *Phyllocnistis citrella* field assay

Phyllocnistis citrella



Lab assays

Experimental methodology



1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* lab assay

Final protein concentration (ug/ml)	*Final volume (ml)	mg powder BLB1	mg powder LIP	mg powder DELFIN
30	20	285.7	360.1	247.1
50	20	476.2	600.2	411.9
70	20	666.7	840.3	576.6
100	20	952.4	1200.5	823.7
150	20	1428.6	1800.7	1235.6



Lethal concentration (LC50)

Experimental methodology



1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* lab assay

	30 ug/ml	50 ug/ml	70 ug/ml	100 ug/ml	150 ug/ml
BLB1	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
LIP	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
DELFIN	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae
CONTROL	10 larvae	10 larvae	10 larvae	10 larvae	10 larvae

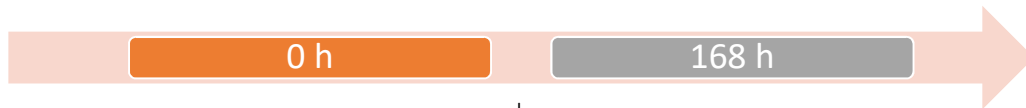
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Lab assay design

Experimental methodology

1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* lab assay



- 10 larvae
 - Apply treatment by spraying method
- Counting the remaining larvae
 - Determine mortality rate

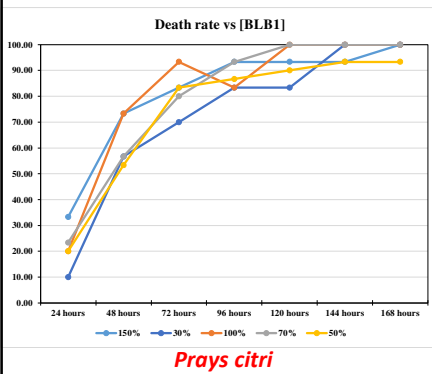


R&D2: Lab assays

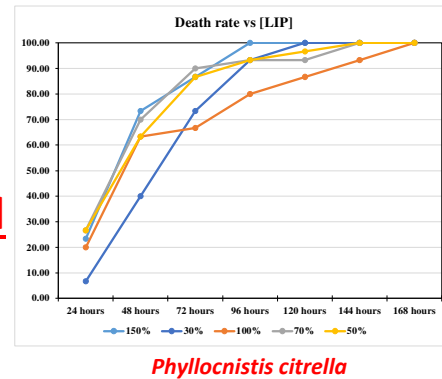
1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* lab assay

After testing the five concentration from 30 µg/ml to 150 µg/ml



LC 50% = 50 µg/ml

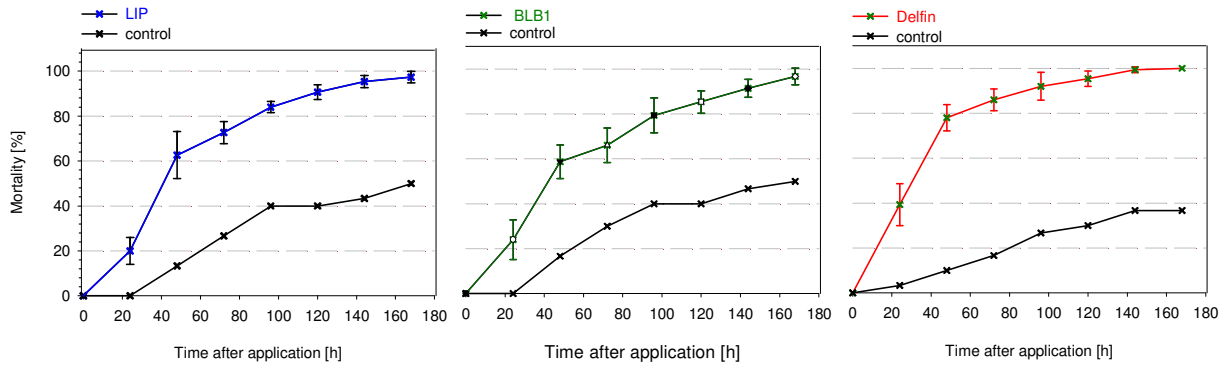


R&D2: Lab assays

1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* and lab assay

Prays citri (lab)

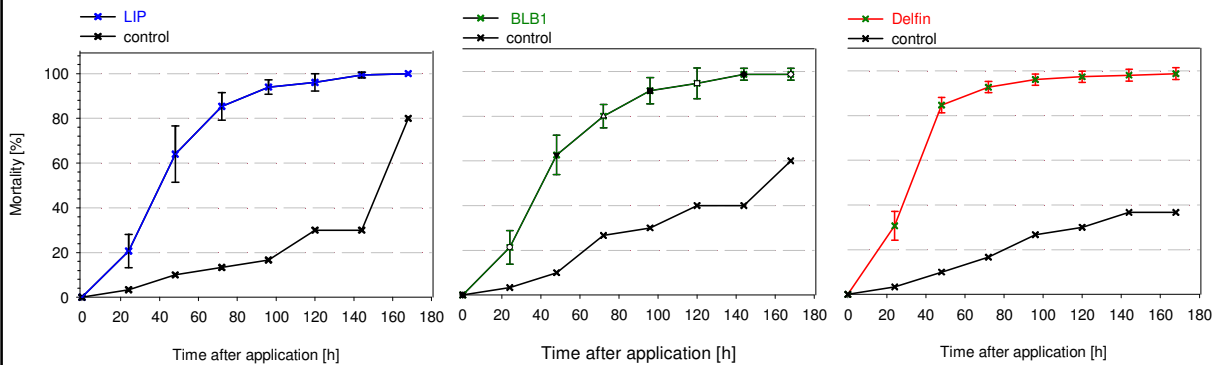


R&D2: Lab assays

1. Efficacy experiments

- *Prays citri* and *Phyllocnistis citrella* and lab assay

Phyllocnistis citrella (lab)





Experimental methodology

2. Spore's persistency test

- Colony forming unit

Step 1

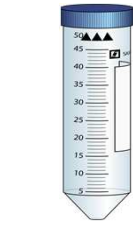
9 leaves per tree for each sampling
 =
 45 leaves per block
 =
 180 leaves per product



Citrus leaves

Washing

Isotonic solution



Spores suspension

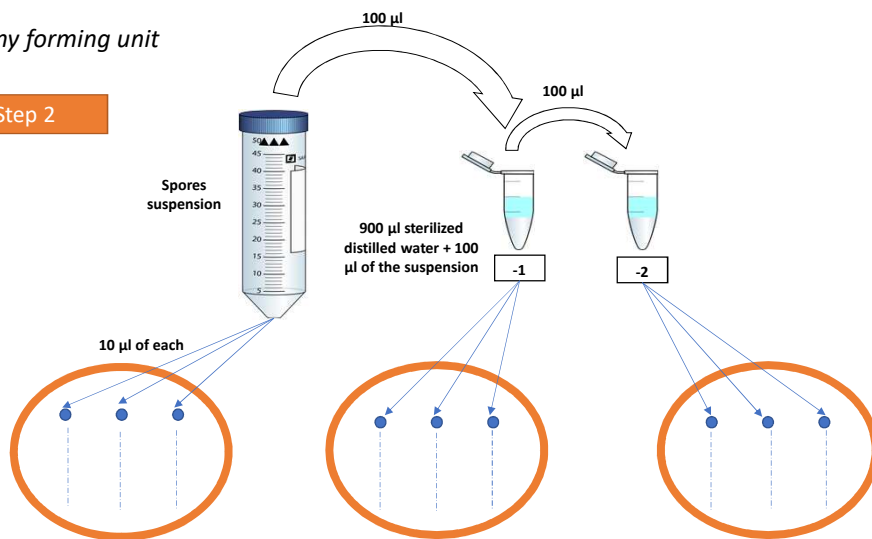


Experimental methodology

2. Spore's persistency test

- Colony forming unit

Step 2



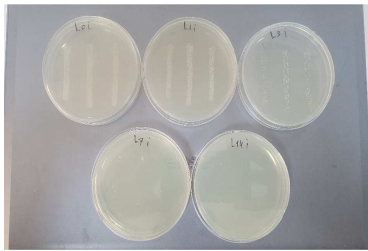
R&D3: Persistency test

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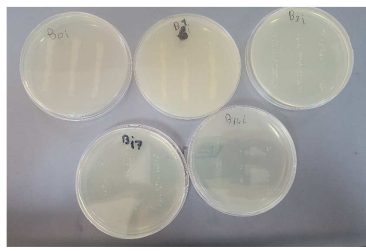
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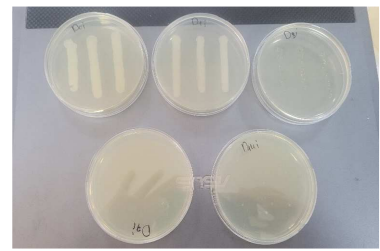
2. Spore's persistency test



LIP



BLB1



DELFIN

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R&D3: Persistency test

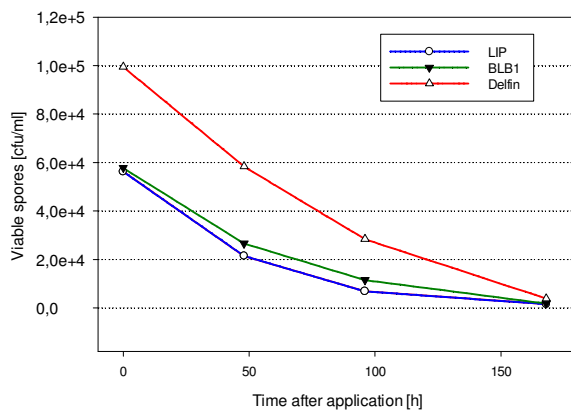
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2. Spore's persistency test

Residues of Bt-spores on the leaf (field)



Spores ↘
through the days

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Conclusion and perspectives

Field assays:

- Our products are **efficient and comparable** with the well known DELFIN
 - ✓ Mortality rate between **50 % - 70 %**

Lab assays:

- Lethal concentration 50 % is determined with **50 µg/ml**
 - ✓ Mortality rate from **80 % up to 100%**

Persistency test:

- No problems with residues are expected.



Conclusion and perspectives

Lab assay targeting another insect model

- *Tuta absoluta*
- *Spodoptera frugiperda*

Justify our products in terms of health, food and environment



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From Research From Lab ...to Market ...to Field



Thank you !



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