Project BIPC (ATCZ00189)

Bioinsecticides for Insect Pest Control

The BIPC project focuses on the:

development of biopesticides,

study of immune responses in insect pests

prospects for the use of RNAi and sustainable methods in insect pest control.

The aim is to study in detail the relationship of the bark beetle, *Lymantria dispar* and Colorado potato beetle with pathogens (bacteria, fungi, nematodes) that could potentially be used for biological control of their populations in the programme area, thus contributing to environmental protection and obtaining more sustainable pest control methods.

Activity 1.1

Ensuring laboratory production of insect pests and selection of their pathogens

Outcomes:

A) *Providing insect material through breeding*- Ensuring the production of insect pests in laboratory conditions

- Ips typographus, (BOKU).
- Lymantria dispar (BOKU)
- Leptinotarsa decemlineata (BC).

B) *Characterization of suitable patogens* - Suitable pathogen strains will be selected according to efficacy, spectrum of action, survival time and resistance to weather conditions.

C) *Provision of live pathogen material through breeding* - Ensuring sufficient quantity and quality of the required pathogens under laboratory conditions..

Activity 1.2

Development and search for a set of functional methods for modification of biopesticides

Outcomes:

A) *Method 1 - Insect gene function - RNA sequencing* - Comparison of immune responses of treated and untreated insects to pathogens using molecular methods - the result will be used to select appropriate genes for silencing the immune responses of pests.

B) *Method 2 - Production of double-stranded RNA* - Bacterial production of specific double-stranded RNA (dsRNA) derived from important pest genes. It will be used for RNA interference, or blocking these genes.

C) *Method 3 - Application of double-stranded RNA into the pest organism* - Finding the ideal formulation for applying dsRNA to the appropriate stage of the pest in the form of a water-soluble (spray), alginate capsule (food) or gel (wood treatment) and monitoring the effect on the expression of blocked genes.

Activity 1.3

- **Laboratory trials of biopesticide application**

- **Outcomes:**
- A) *Validated technology for Leptinotarsa decemlineata (BC*) - Targeted and ecologically acceptable control of potato beetle in agriculture without harming non-target species and without damaging ecosystems.
- B) *Validated technology for Ips typographus, (BOKU)* - Targeted and ecologically acceptable control of spruce bark beetle in forestry with minimal effect on non-target species.
- C) *Validated technology for Lymantria dispar (BOKU)* - Targeted and ecologically acceptable control of *Lymantria dispar* in forestry without damaging non-target species and ecosystems.

Activity 1.4

- **Monitoring the effects of innovative biopesticides under field conditions (semi-field conditions)**

- **Outcomes:**
- A) *Studies on the effects of innovative biopesticides* - A document reflecting the laboratory and semi-field results of the use of the bioinsecticide, including the environmental impact, which will include the outputs of activities 1.2 to 1.4.
- B) *Modified prototype biopesticide for individual insect pest species* - A biopesticide prototype targeting a specific pest, a specific stage of development with an optimised form of application.

Activity 1.5

- **Dissemination of project results and outputs to target groups**

- **Outcomes:**
- A) *Workshops and student conferences* - BC will organize a series of one-day workshops for the general public and professionals with a capacity of about 20 people/workshop.BOKU will organize student conferences once a year at its department, where the results of the project will also be presented.
- B) *Educational materials for target and stakeholder groups* - Information and promotional materials for professional groups and the public - approx. 220 copies (200 Czech version, 20 German version);articles in professional periodicals (e.g. Zahrádkář, Agromanual, Rostlinolékař, Forstschutz Aktuell, Österreichische Forstzeitung, etc.)
- C) *Professional publications including the results of the project* - Three scientific articles in peer-reviewed journals reflecting the results of the project, together with an Open Access upload

Progress report

Activity 1.1 - we have established laboratory production of insect pests, we are working on the characterization and production of patogens (BOKU + Marija + Oxana + Jirka + Daniela + Vláďa)

Activity 1.2 – We have performed RNA sequencing as planned – we have received transcriptomes, which are useful… (Michal, Martina). We also produce dsRNA and optimize its use (Vašek + students).

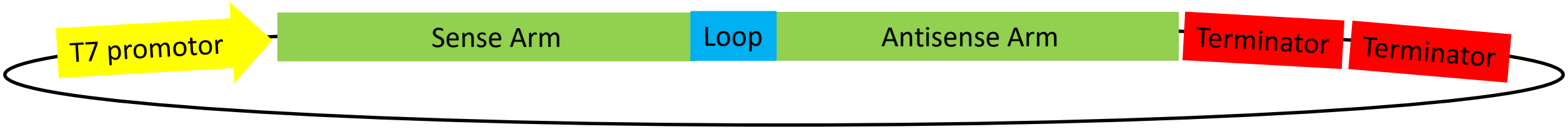
Activity 1.3 – laboratory tests – the preliminary results look promissing (Vašek, Oxana, Maja, Marija, Martina)

Activity 1.4 – Semi-field results – putting together the planned document?

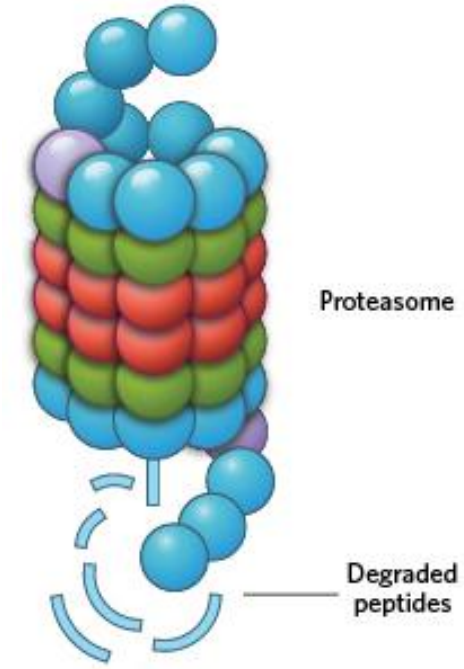
Prototype biopesticide – Jirka

Activity 1.5. Dissemination – workshops – Jirka, publications

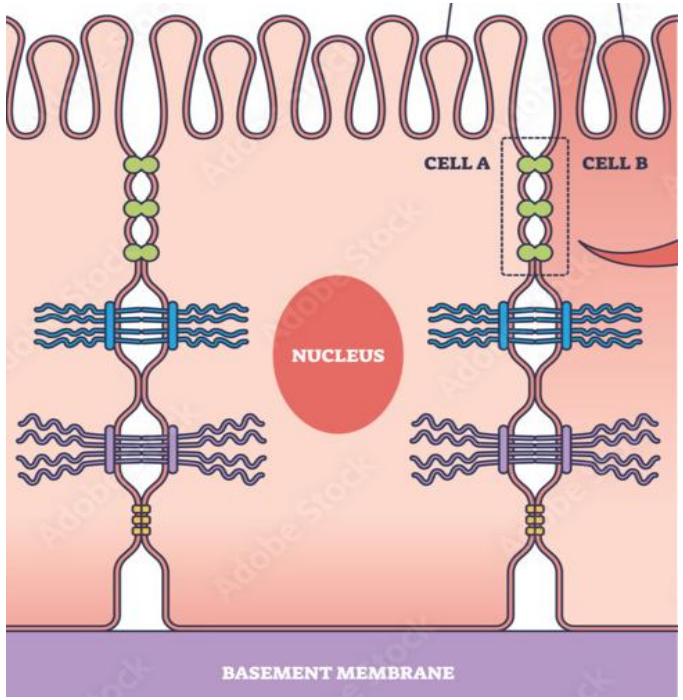
dsRNA Hairpin construct



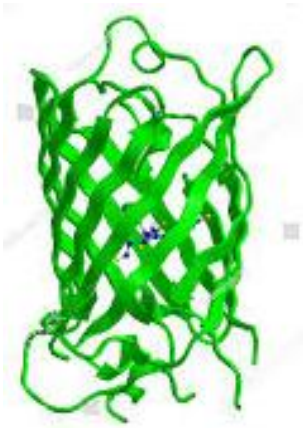
Proteasome subunit Beta 5
„Ledprona“



midgut junction protein Mesh



Green Fluorescent protein (GFP)



Hairpin construct

„Ledprona“

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GFP

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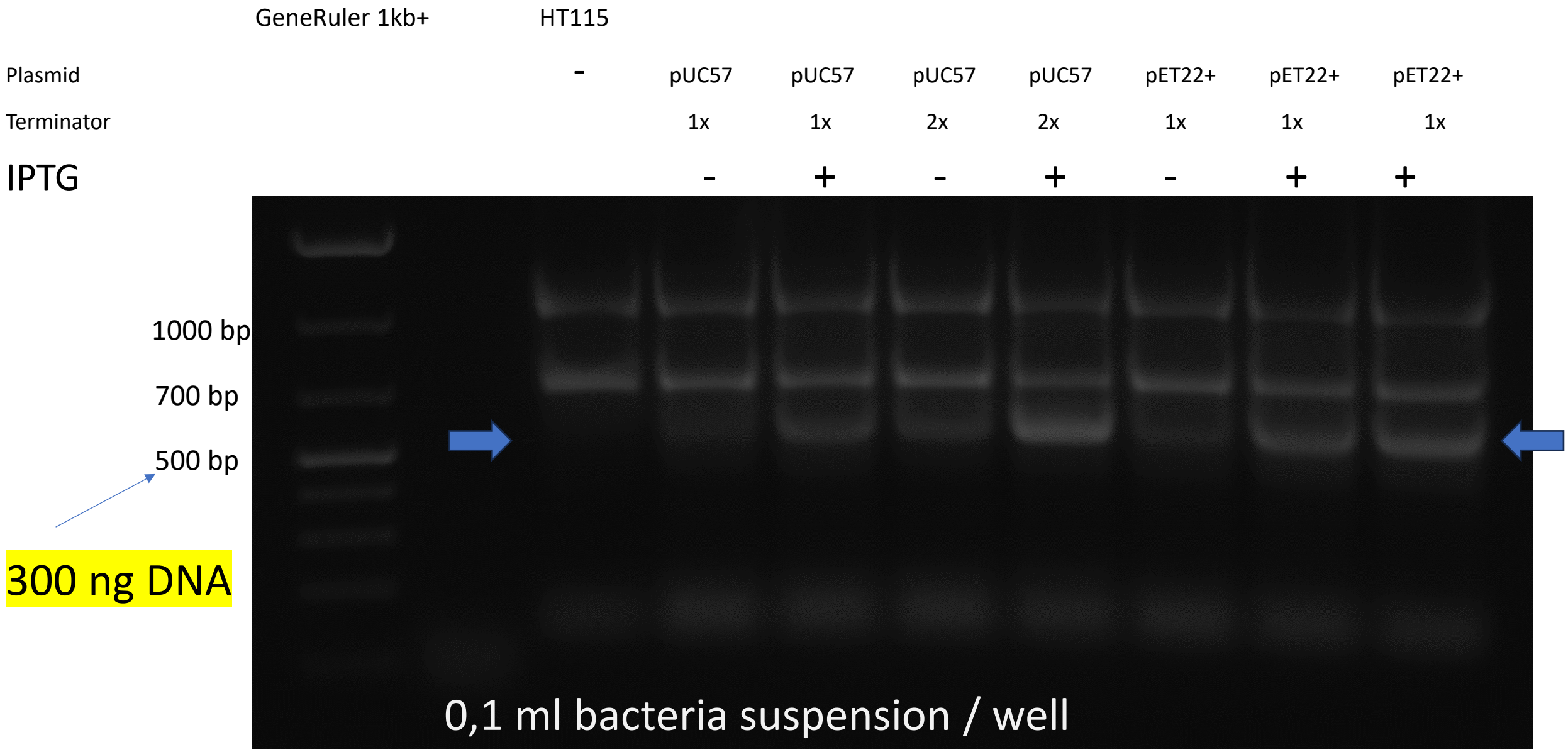
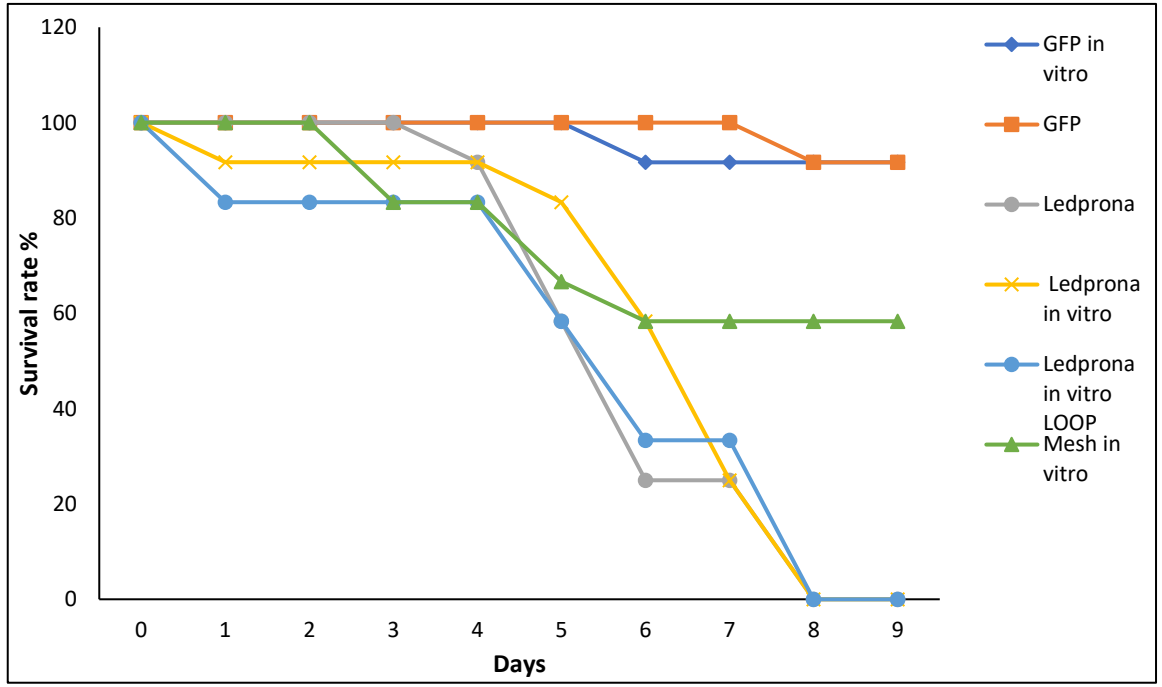
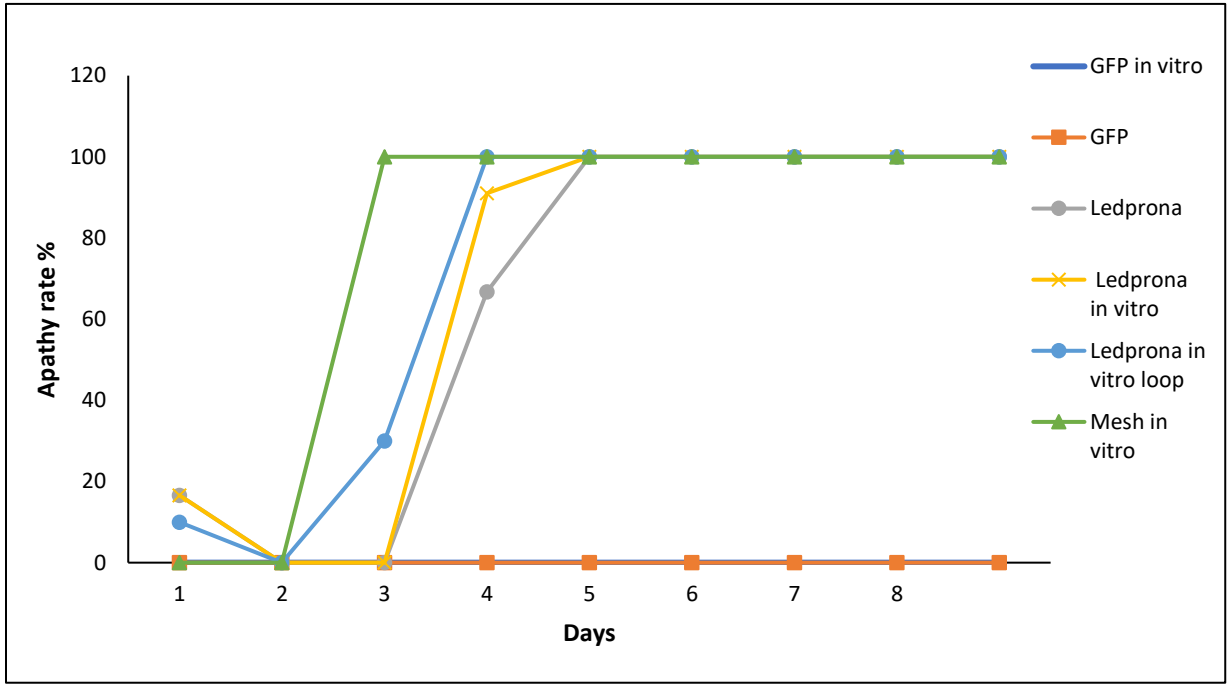
dsRNA isolation from *E. coli* HT115 (0,5 mM IPTG, 6 hrs induction)



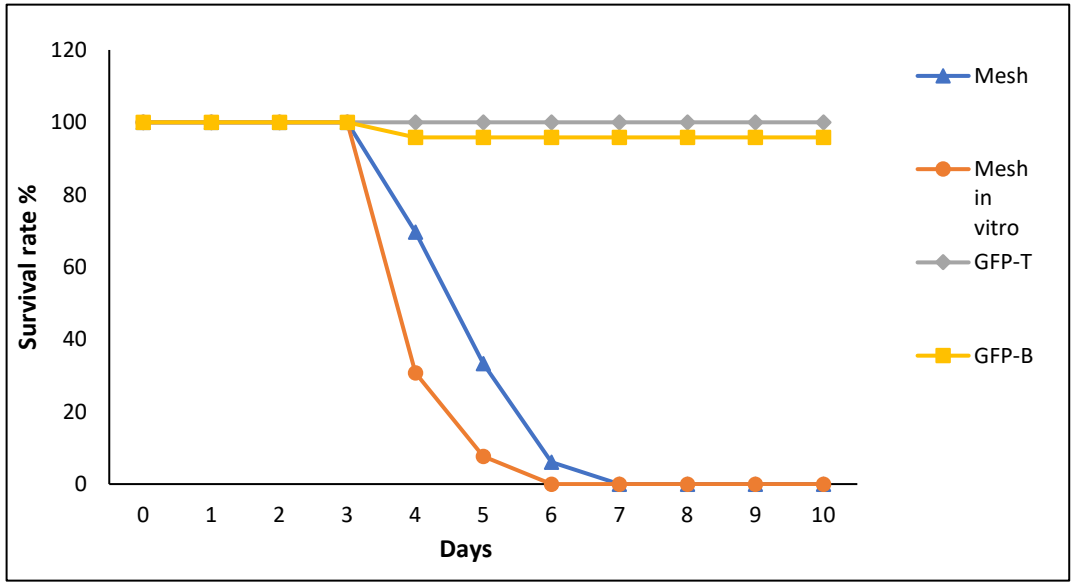
dsRNA feeding trials – adults potato beetles

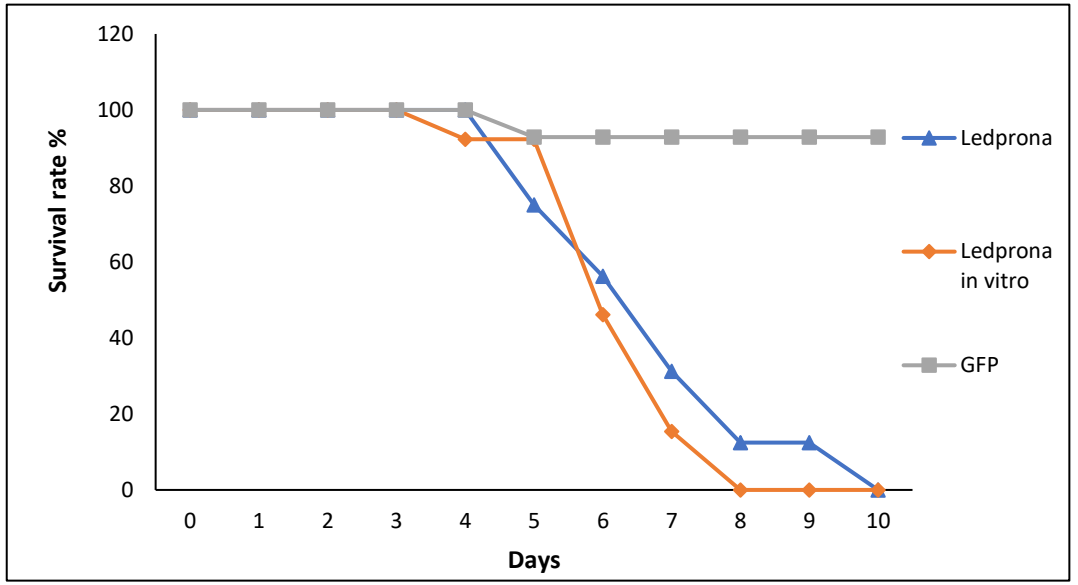


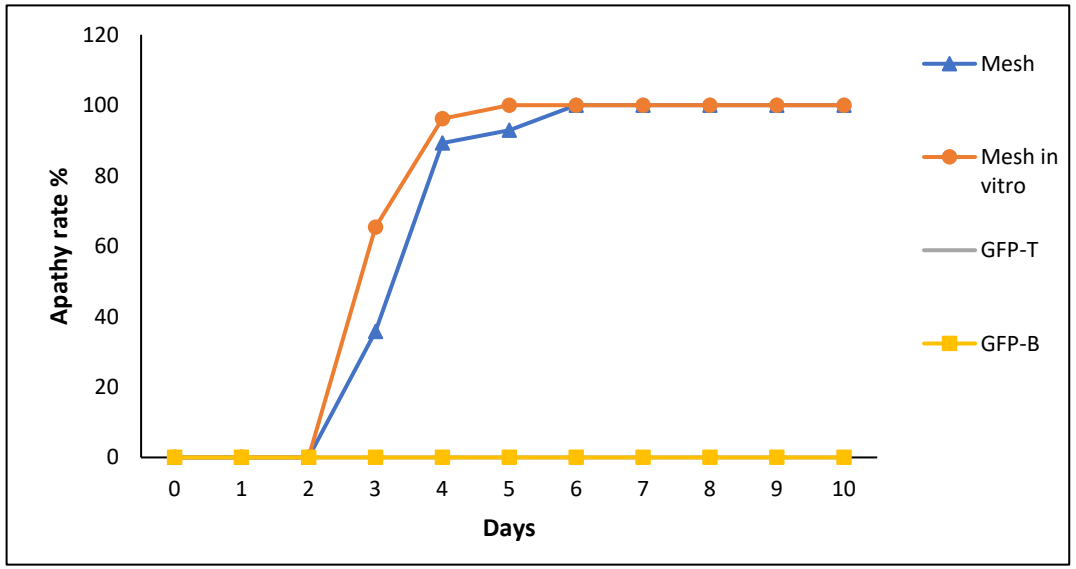


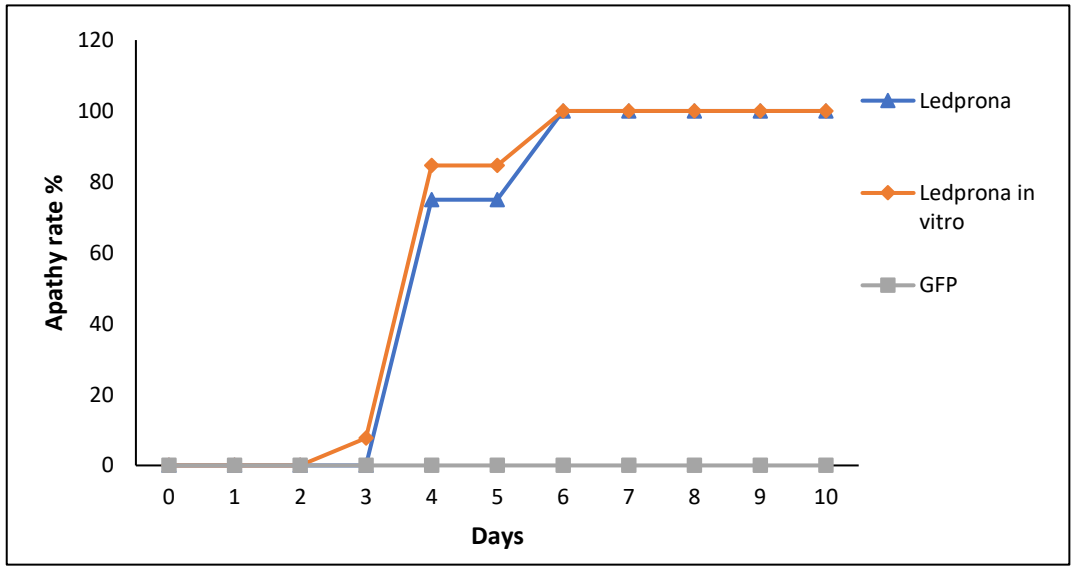
dsRNA was prepared from Leptinotarsa genes - proteasome subunit (ledprona), Mesh (cell to cell adhesion protein) and GFP (control)

Feeding trials – larvae (II., III. instar)

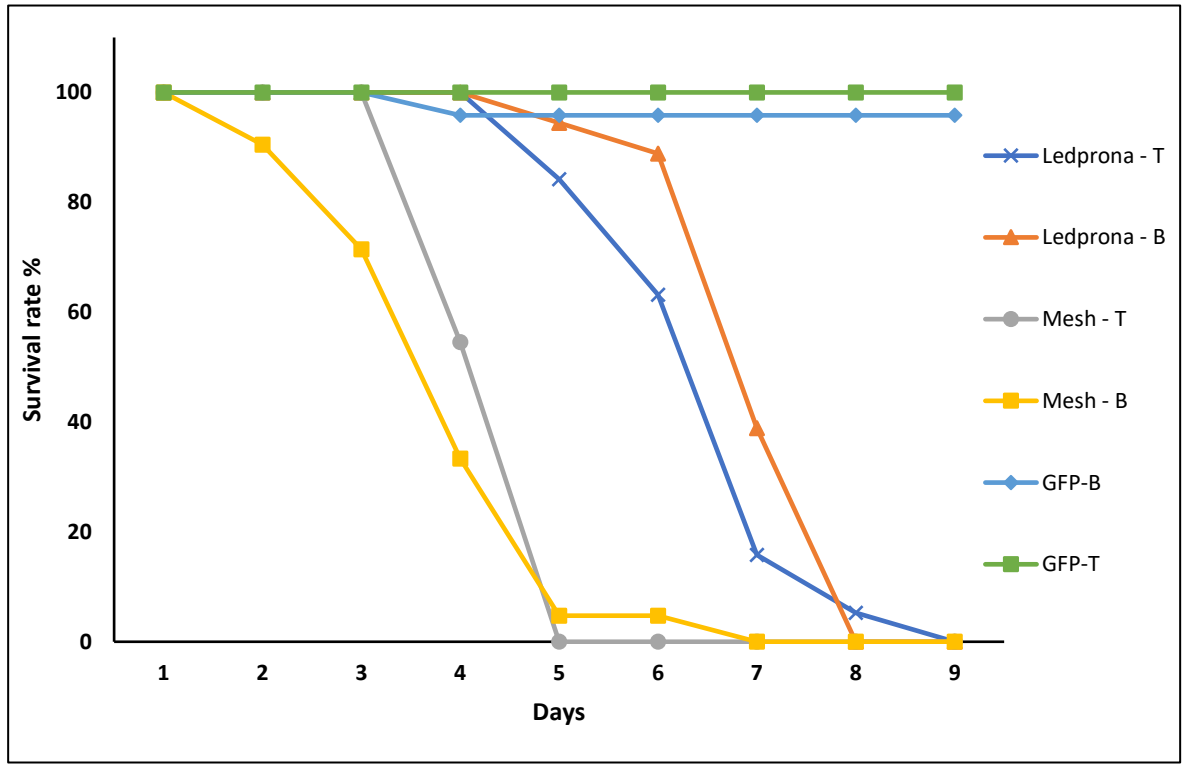


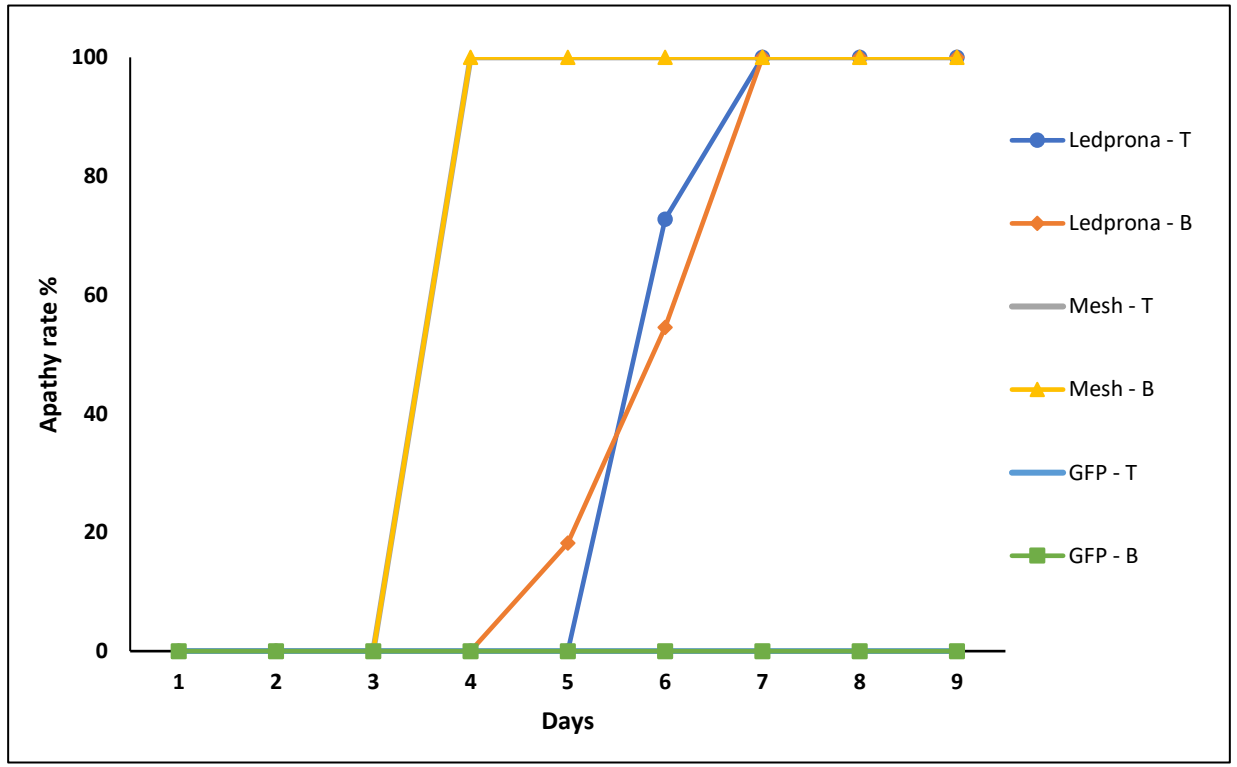






Feeding trials – larvae (II.instar)





Leptinotarsa – dissection of guts from treated adults

GFP

Mesh – in vitro

Mesh- specific dsRNA causes problems in proventriculus (full), the food does not go to other parts of gut.