Development and production of novel fusion protein biopesticides

Researching biological alternatives to chemical pesticides

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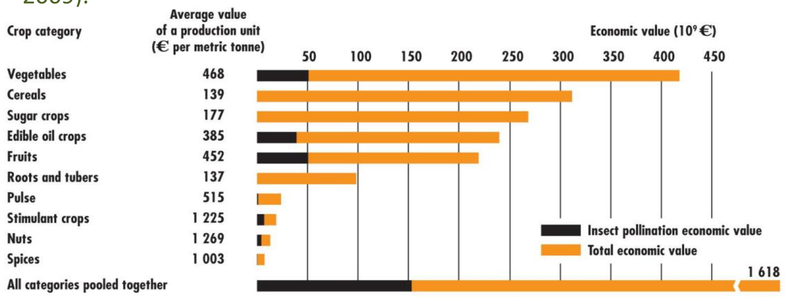
INTRODUCTION

The use of common chemical pesticides particularly neonicotinoids poses unacceptable eco-agronomical risks due to declines in pollinator populations (Figure 1) and studies on pollinators such as honey (Henry et al, 2012) and bumble bees (Whitehorn et al, 2012) indicate declines in queen numbers, learning and memory defects, increases in numbers that don't return to the hive and ultimately colony-collapse disorder.

FIGURE 1: Economic significance of insect pollination on agricultural production of human food crops worldwide (UNEP 2010; Gallai et al 2009).



The contribution of pollinators to the production of crops used directly for human food has been estimated at €153 billion globally, which is about 9.5% of the total value of human food production worldwide [6].

The aim of this project was to expand on research by the Gatehouse group by developing novel GNA fused biopesticides derived from the SFl family of neurotoxic proteins from the venom of the tube web spider *Segestria florentina* (below), and express them in the yeast *Pichia pastoris.*

The proteins induce flaccid paralysis in insects and larvae, acting on voltage-dependent calcium (CaV) channels (Fitches et al, 2004).

The insecticidal protein is fused with snowdrop lectin (GNA -*Gallanthus nivalus* agglutinin) so that upon ingestion, the protein is absorbed through the mid-gut and into the insect haemolymph.

A similar biopesticide using ω-hexatoxin-Hv1a from the Australian funnel web spider *Hydronyche versuta* fused to GNA has been shown to have minimal adverse effects on honeybees following injection or chronic consumption (Figure 1; Nakasu et al, 2014) but also exhibits potent toxicity on a range of insect orders including Lepidoptera, Coleoptera, Diptera and Heliptera.

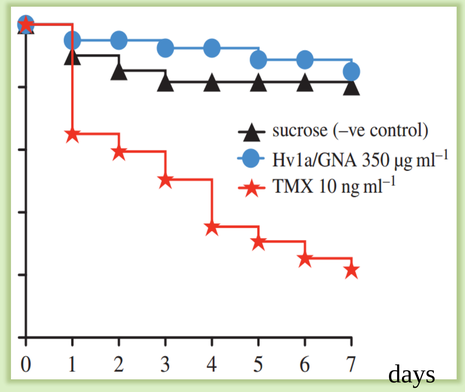


FIGURE 2: Chronic consumption of Hv1a/GNA (21.7µg bee $^{-1}$day^{-1}) does not affect honeybee survival.

Thiamethoxam/TMX – a neonicotinoid, (0.727ng bee $^{-1}$day^{-1}) significantly increases mortality.

(Taken from: Nakasu et al, 2014).

AIMS & METHODS

Project A: Develop and produce biopesticides from the family of eight *S. florentina* insecticidal toxins (SFL1-8).

1. **Cloning the toxin DNA:** PCR Toxin DNA → Ligated into pCR2.1 plasmid vector →Transformed into *Escherichia coli* →Blue-white selection to select positive transformants → Positives grown overnight → Plasmid harvested.

2. Expression in *Pichia pastoris:* Digested recombinant pCR2.1 plasmid with restriction enzymes (Not1 and Xho1) →Digested pGAPzα vector (yeast integrating plasmid) containing the GNA sequence with same restriction enzymes → Toxin sequence and pGAPzα ligated together → Transformed into *E.coli* and cloned → Transformed into *P. pastoris* for expression of biopesticides.

Project B: Develop two novel synthetic SFl toxins by substituting amino acids from variable regions of one toxin with amino acids from the variable region of another in the same family.

The DNA encoding a synthetic toxin fused with GNA was synthesised by a two-step, 'templateless' PCR assembly of a series of overlapping oligonucleotides.

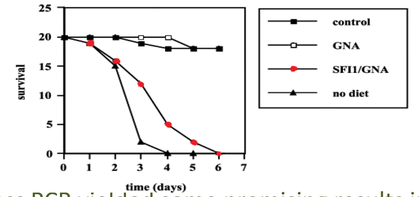
The toxin DNA is then transformed, cloned and expressed in much the same way as in Project A (above) with one exception: Since the synthetic sequence encoded GNA, the pCR2.1 and pGAPzα vector were restricted using Xba1 rather than Not1 when preparing to transform into *P. pastoris.*

RESULTS & DISCUSSION

Project A

- Successfully cloned all eight SFl toxins in *E.coli* using the pCR2.1 plasmid vector, and transformed four of the toxins into the pGAPzα vector system.
- SDS-PAGE suggested we successfully expressed three different Segestritoxin/GNA fusion proteins in *Pichia pastoris;* SFl1/GNA, SFl3/GNA, and SFl6/GNA (See Figure 4). Further study required to confirm this assumption.
- Previous studies indicate SFl toxin/GNA fusion protein genes are potential candidates as biopesticides and show acute toxicity when fed to larvae of tomato moth, *Lacanobia oleracea (*Figure 3; Fitches et al, 2004).

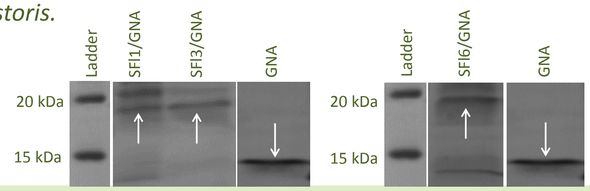
FIGURE 3: SFl1/GNA has acute toxicity on *Lacanobia oleracea* larvae 6 days after ingestion (Taken from: Fitches et al, 2004).



Project B

- The templateless PCR yielded some promising results initially, however we were unable within the timeframe to create the synthetic SFl toxins; with more time we would have been successful since whole synthetic yeast chromosomes have been assembled using a similar protocol (Team JHU, iGEM, 2009).

FIGURE 4: SDS-PAGE showing expression of three SFl/GNA biopesticides in *Pichia pastoris.*



CONCLUSIONS / FURTHER STUDY

- New library of biopesticide candidates for expression in *P. pastoris* and testing for toxicity in important plant pests and pollinator species.
- Optimisation of transformation efficiency and protein expression required for improved productivity of fusion proteins.
- More time required to optimise the oligonucleotide assembly of synthetic toxin/GNA fusion proteins.

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PICTURES

Tube Web Spider, *Segestria florentina* photograph
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