Control of cyst nematodes using egg hatching compounds

Yogendra Gaihre
ykg1n16@soton.ac.uk

Supervisors;

Lindy Holden-Dye & Vincent O'Connor, University of Southampton
Vivian Blok and Wayne Morris, James Hutton Institute

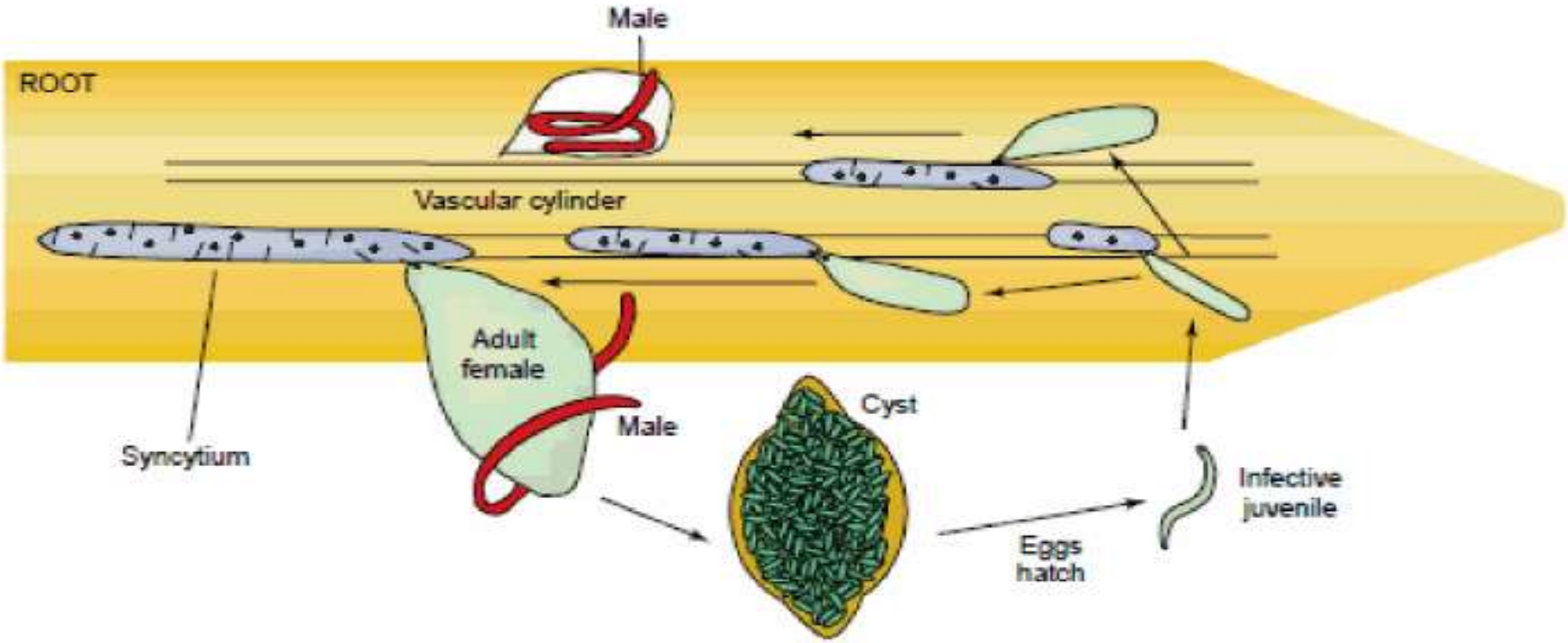
The problem of potato cyst nematodes (PCNs)

- Potato cyst nematodes (PCNs) *Globodera pallida* and *G. rostochiensis* are economically important pests of potatoes worldwide.
- PCNs cause losses of 9% of total potato production worldwide (Turner and Rowe, 2006).
- In the UK loss is estimated at around £50 million per annum (www.cabi.org).
- In Scotland, data collected by SASA show a steady increase in the incidence of *G. pallida* in statutory PCN tests over the past 25 years.
- Once established they persist for long periods.
- Changes in legislation regarding chemical use exacerbates the threat.

The James Hutton Institute

UNIVERSITY OF Southampton

Life Cycle of PCN



Hatching in Potato cyst nematode

Eggs in Potato root exudates

Eggs in water

The James Hutton Institute

UNIVERSITY OF Southampton

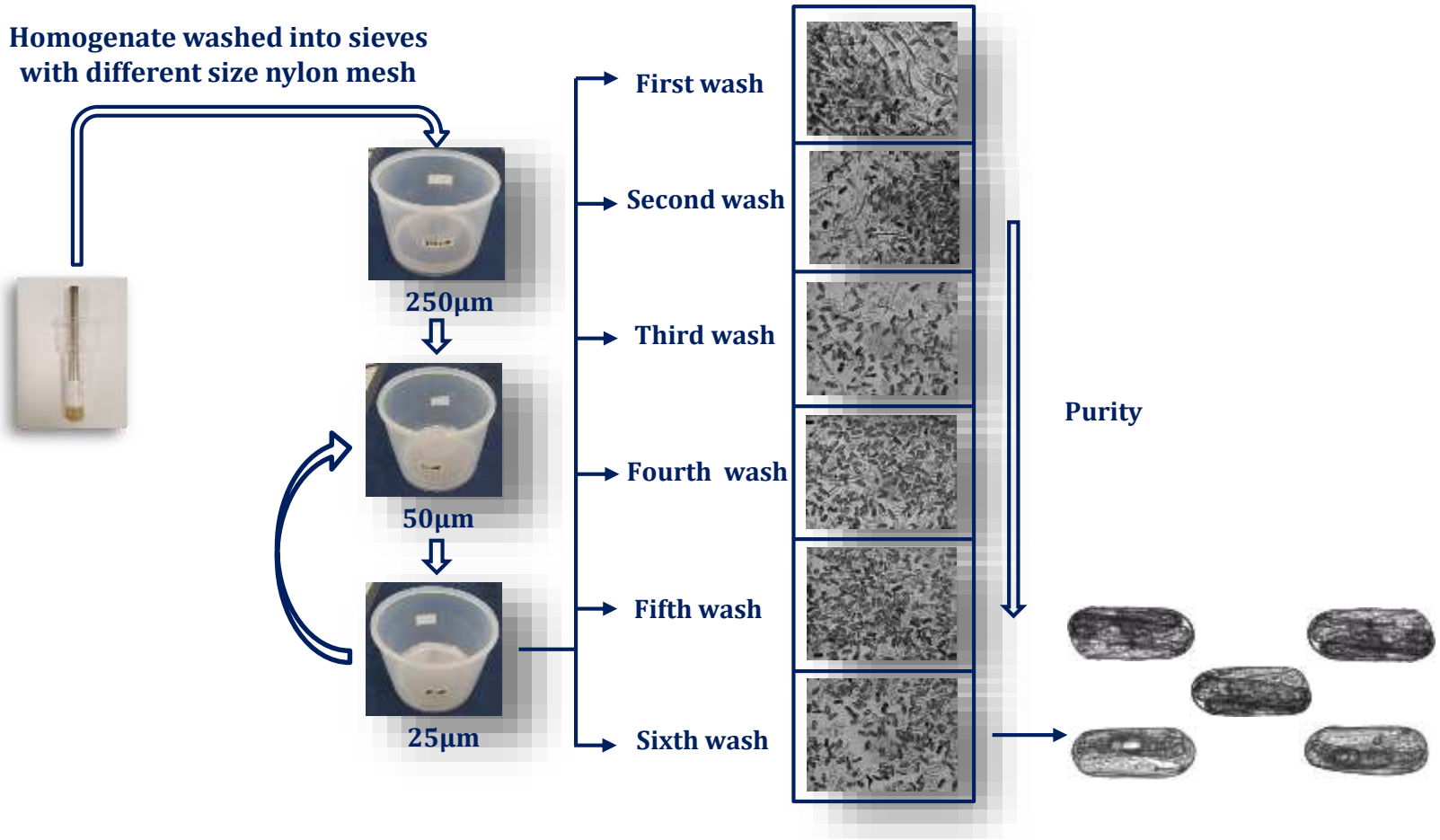
Aims of the study

- Optimize *in vitro* hatching assays for *Globodera sp*.
- Identify hatching factors from different potato cultivars
- Use this to select potato genotypes with low level of hatching factors that can be used for breeding for PCN non- host cultivars
- Candidates genes that regulate the production of hatching factors can be used in the future to use a synthetic biology approach to produce large amounts of hatching factors in artificial expression systems

The James Hutton Institute

UNIVERSITY OF Southampton

Optimizing *in vitro* hatching assays



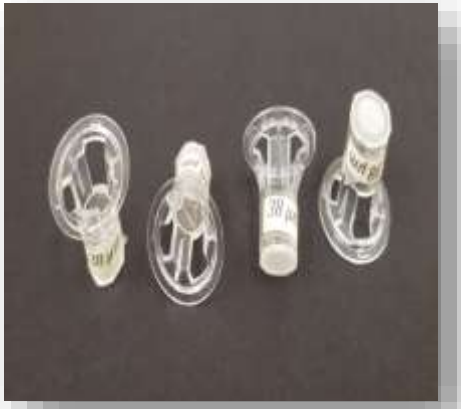
The James Hutton Institute

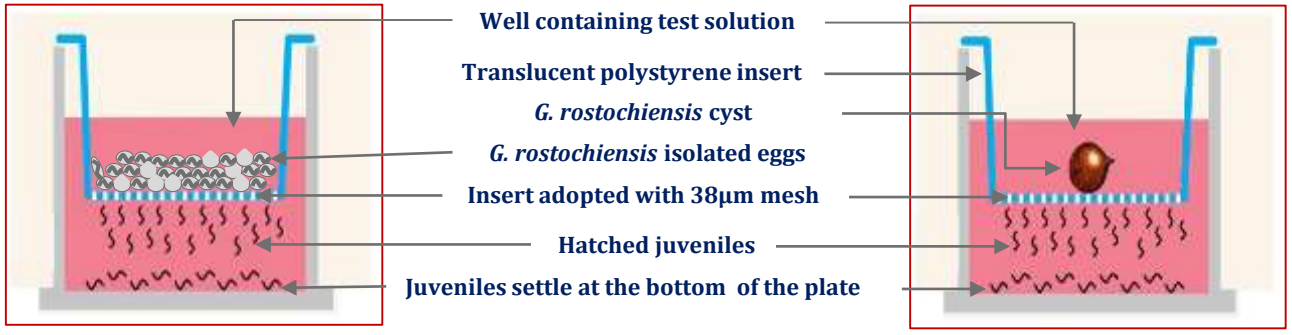
UNIVERSITY OF Southampton

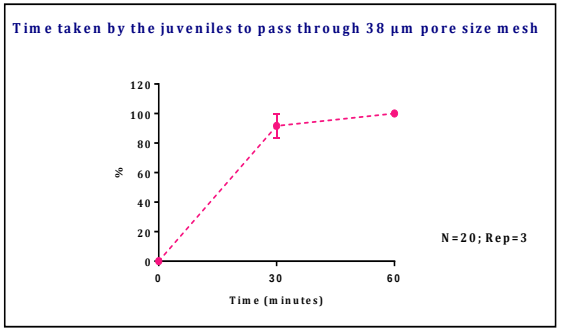
Design of a hatching device for isolated eggs

Hatching Devices

- Made up of translucent polystyrene materials (COSTAR)
- Translucent inserts with 6.5mm diameter
- Bottom outer diameter : 9mm
- Bottom inner diameter : 7mm
- Insert height : 18 mm
- Insert depth : 9mm that can hold 300 µl of liquid
- Inserts are adapted to have 38-µm pore size nylon mesh.



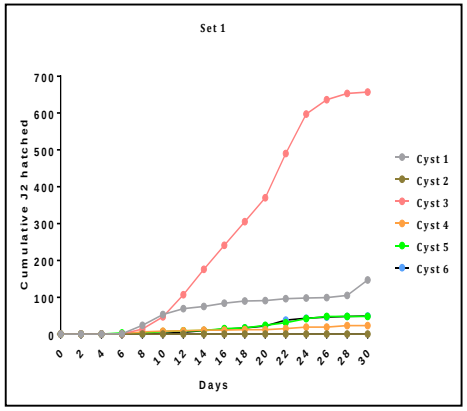


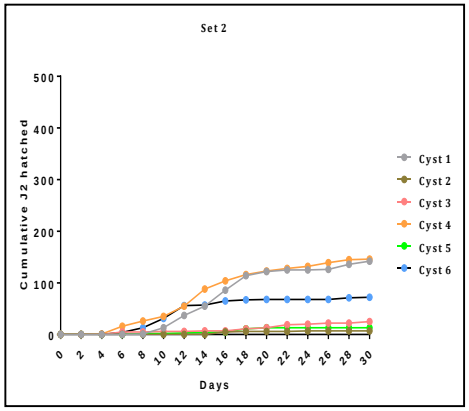


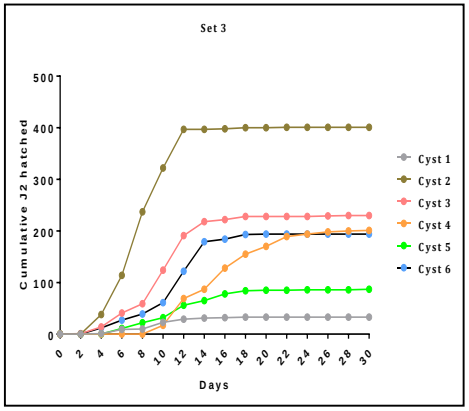
The James Hutton Institute

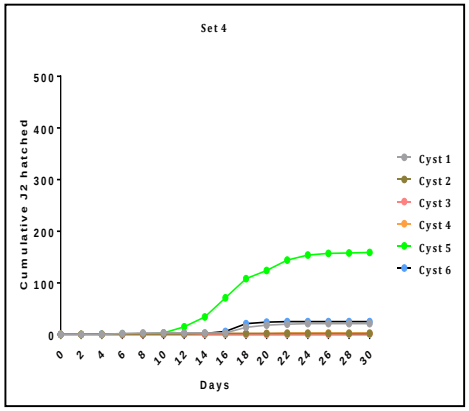
UNIVERSITY OF Southampton

Cyst shows HIGH variability

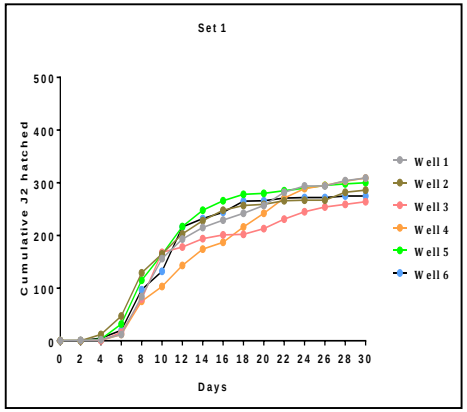


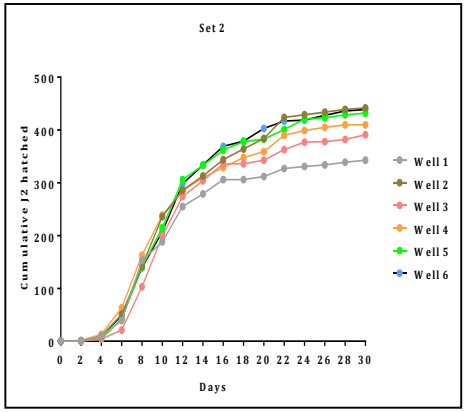


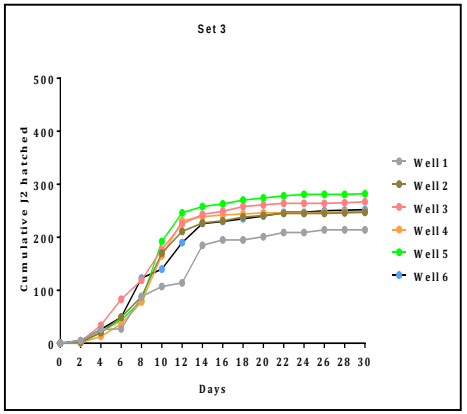


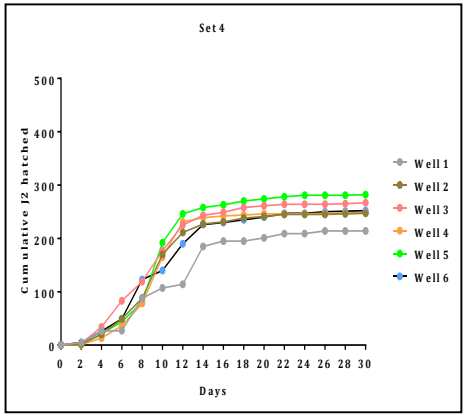


Eggs shows LOW variability









The James Hutton Institute

UNIVERSITY OF Southampton

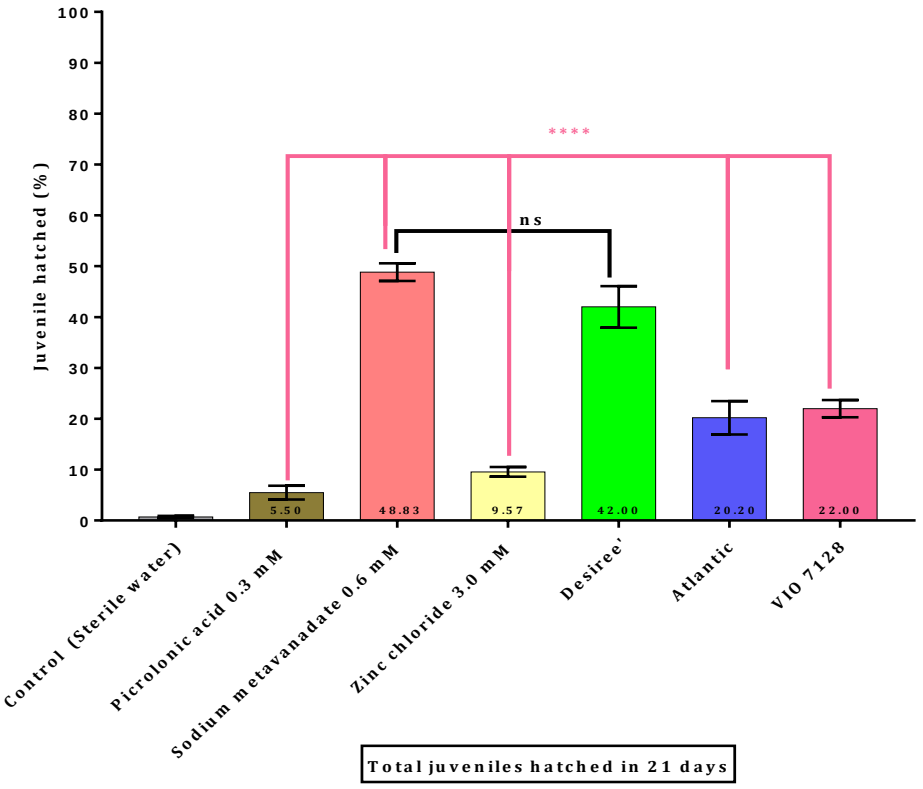
Providing a benchmark for the efficacy of root exudates

- Literature search for chemical activators of hatching
- Testing chemicals for hatching efficacy using the isolated egg preparation
- Optimization of sodium metavanadate as an artificial hatching factor

The James Hutton Institute

UNIVERSITY OF Southampton

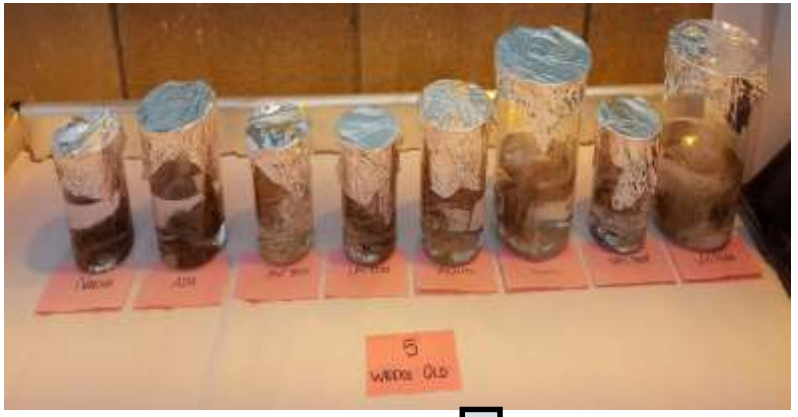
Juvenile emergance from isolated eggs of *Globodera rostochiensis* exposed to different normalised PRE and artificial hatching factors (NORMALISED & DILUTED IN STERILE WATER) POOLED



The James Hutton Institute

UNIVERSITY OF Southampton

Identifying hatching factors from different potato cultivars



Step 1

Quantify hatching efficacy of root exudates prepared in a standardised manner relative to sodium metavanadate

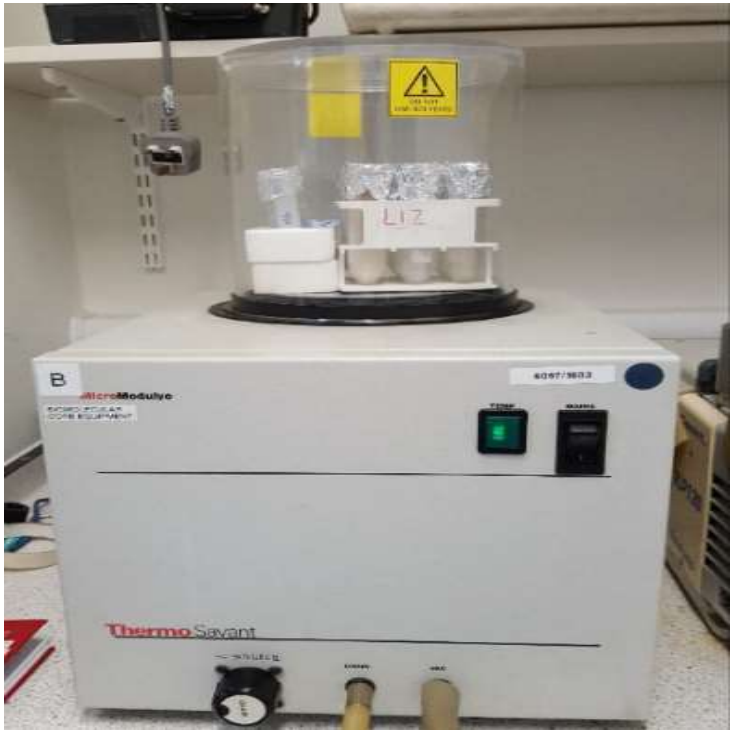
The James Hutton Institute

UNIVERSITY OF Southampton

Identifying hatching factors from different potato cultivars

Step 2

Concentrate samples of root exudate by lyophilisation (Freeze- drying)



The James Hutton Institute

UNIVERSITY OF Southampton

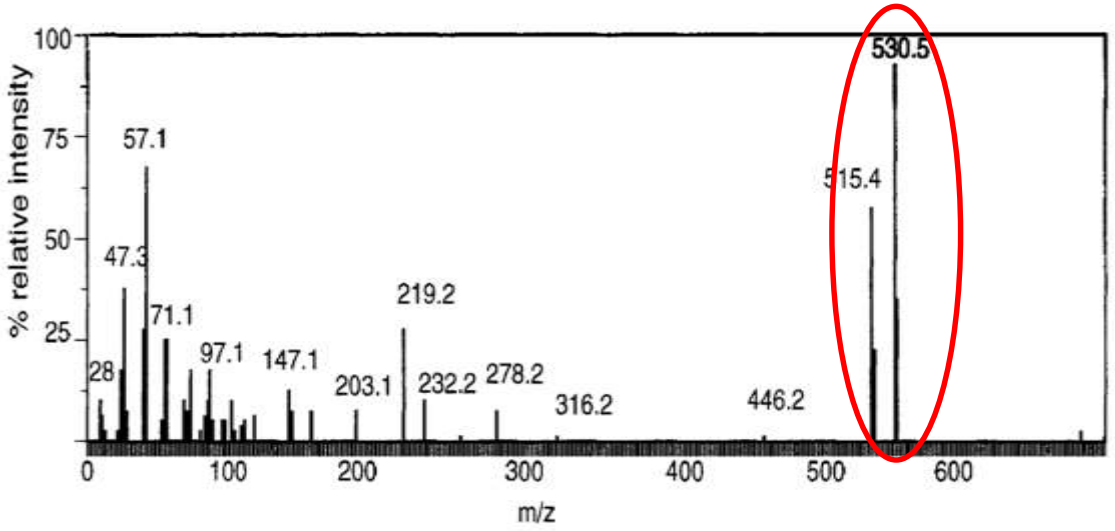
Identifying hatching factors from different potato cultivars

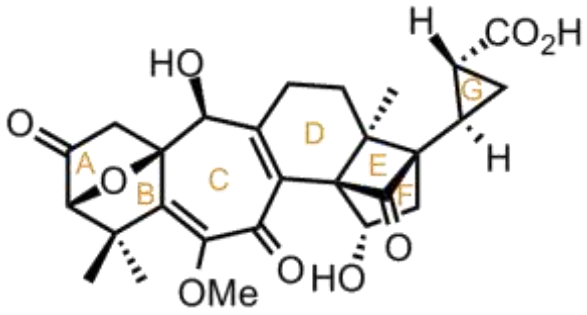
Step 3

Phyto chemical characterisation of root exudates using

High Performance Liquid Chromatography (HPLC)

Mass Spectrometry



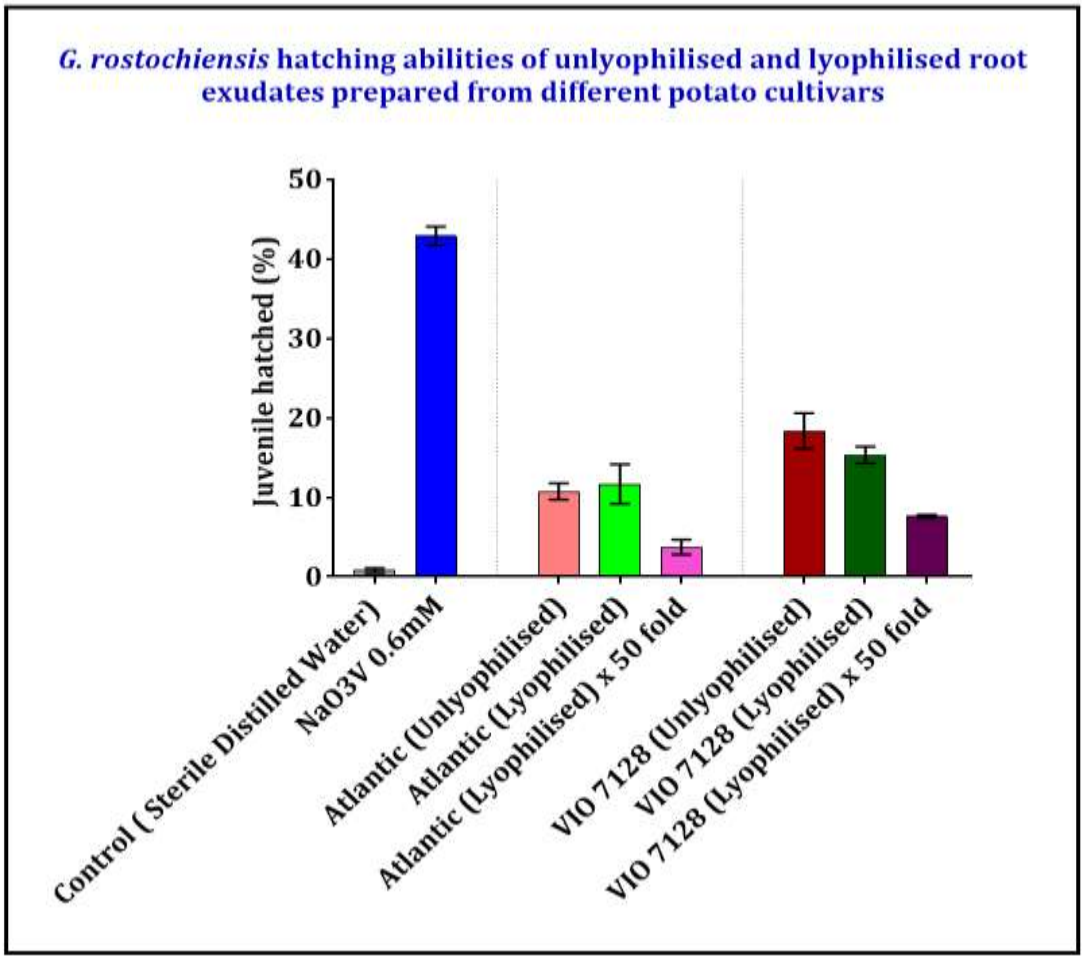


Solanoeclepin A

The James Hutton Institute

UNIVERSITY OF Southampton

Hatching assay with lyophilised samples

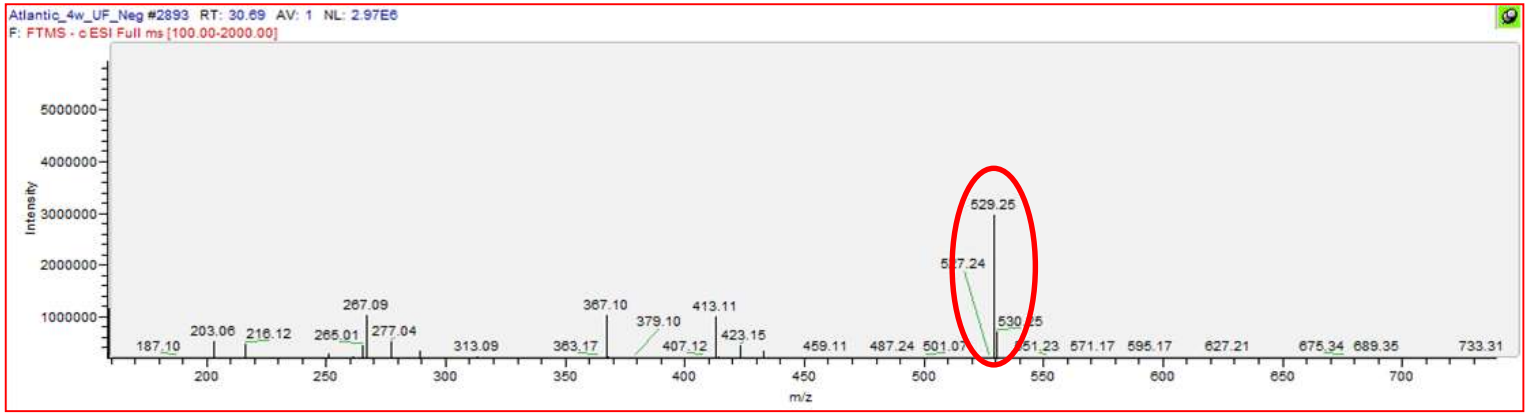


The James Hutton Institute

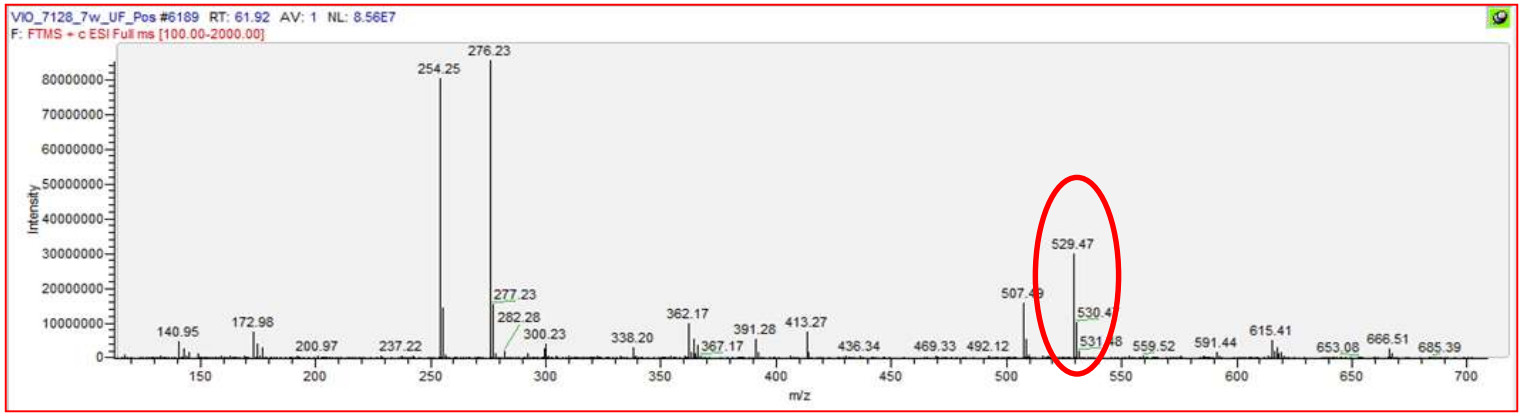
UNIVERSITY OF Southampton

Phytochemical analysis using HPLC and Mass Spectrometry

Solanum tuberosum 'Atlantic'



Solanum violaceimarmoratum



The James Hutton Institute

UNIVERSITY OF Southampton

Summary

An isolated egg preparation is optimal for characterisation of hatching factors

Sodium metavanadate provides a benchmark against which to assay root exudates from different cultivars

Root exudate efficacy is maintained in a freeze dried sample

The James Hutton Institute

UNIVERSITY OF Southampton

Future work

Subject root exudate samples to phytochemical analysis

Pursue this for different potato cultivars with different susceptibilities to PCN infection

Use the isolated egg assay and the prototype hatching device to develop a high throughput approach for screening root exudate for hatching efficacy

Acknowledgements

Funded by;

Scottish Society for Crop Research

Felix Cobbold Trust

James Hutton Institute

University of Southampton