Project report for SSCR-Potatoes

***Project title*:**

Can wounding by free living nematodes allow entry of *Pectobacterium atrosepticum* into potato roots leading to systemic infection and blackleg disease.

Applicant(s):

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Background to the project

Pectobacterium and Dickeya species are plant pathogenic bacteria causing blackleg disease of potato plants and soft rot of potatoes. In the UK, blackleg is caused mainly by *Pectobacterium atrosepticum* (Pba). Although blackleg is primarily a seedborne disease the initial source of contamination of high grade seed is almost certainly environmental. Therefore, understanding the ecology and movement of Pba from soil to roots and aerial parts of the plants is of significant importance. Previous glasshouse studies revealed that both *Dickeya solani* and *Pectobacterium carotovorum* subsp. *brasiliense* are capable of penetrating the roots of potato plants from a soilborne inoculum and moving systemically from the roots to the stem. Our preliminary studies have shown that although Pba is able to penetrate the surface of potato roots we have, so far, not been able to visualize them moving into the xylem and dispersing systemically to the stem. Free living nematodes (FLN) are abundant in agricultural environments and may play a role in the initial entry of soil borne bacteria into roots. FLN have stylets which they use to feed on roots, and a combination of the repeated thrusting of the stylet on the cell surface to penetrate the cell and digestive enzymes generate wounds that may serve as entry points for Pba.

Aims and objectives

To investigate whether the wounds generated by FLN when feeding on potato roots serve as entry points for Pba into the roots and allows access to the xylem. The basic function of the xylem is to transport water from the roots to the aerial part of the plant so entry to the xylem could allow Pba to move systemically from the roots to the stem. Therefore, we would like to visualise potential dispersal of Pba from the xylem in the roots to the stem using confocal microscopy of microplants grown hydroponically and selective plating of plants grown in compost in the glasshouse.

Research results

The aim of this project was to identify whether FLN in the soil are facilitating the movement of environmental Pba into the roots of potato plants and providing Pba with access into the aerial parts of potato plants. *Solanum tuberosum* cv. *Estima* were inoculated with Pba1039 tagged with Green Fluorescent Protein (GFP), and a community population of nematodes were introduced to the soil by pouring the suspension into 3 holes in the soil around the plant base. A 38µm mesh was placed at the bottom of the pots to prevent the nematodes from escaping from the soil. At 4 weeks post-inoculation the plants were harvested and separated into roots and stems. Plant parts were cleaned with ethanol and bleach to ensure that only internalised bacteria remained and, to ensure the cleaning step was effective, imprints of the plants were taken on LB + ampicillin plates before and after cleaning. The plant parts were then homogenized, serial dilutions were made and plated onto crystal violet pectate (CVP) agar (a semi-selective media for Pba) in duplicate. Plates were incubated at 27°C for 2 days and after this time any colonies causing dips in the CVP media were counted and checked under a blue light for GFP expression. The results from this experiment indicate that nematodes do contribute to the internalisation of Pba into potato plants, with consistently higher numbers of colonies detected from plants co-inoculated with Pba and nematodes compared with the Pba only controls (Figure 1). The results show a 10-fold increase of Pba in roots and a 100-fold increase in stems when nematodes are present. This experiment was repeated, but unfortunately was unsuccessful due to no colonisation of the roots occurring. We are unsure of the reason for the bacteria not colonising the roots and therefore this experiment is currently being repeated to confirm results.

In addition, we also used confocal microscopy to determine if we could visualise systemic movement of Pba from the roots to the stem of the plant and whether the addition of nematodes aided systemic movement by allowing entry of the bacteria to the xylem. A hydroponics system comprising of a micro-propagated potato plant with roots dipping into plant growth medium was used to study the colonisation of potato by either Pba or Pba inoculated with nematodes. When plants were only inoculated with Pba, bacteria could be observed accumulated in cells of the epidermis or cortex but were never observed in the xylem. When Pba was co-inoculated with a range of free-living nematodes, the bacteria penetrated to deeper cell layers within the root. However, the xylem still appeared uncolonised and although bacteria were located close to the base of the crown, they could not be detected above the crown in the stem tissue (Figure 2). This result does not appear to represent the results we saw in the above experiment when using fully grown plants in the glasshouse and therefore we are working to adapt the microscopy method by using larger plants grown from mini-tubers in sterile growth media.

Outcomes

Determine whether systemic colonization of potato plants from infected soil through wounds generated by nematode feeding could play a significant role in the epidemiology of potato blackleg caused by Pba.

From the results above it does appear that wounding by FLN could play a role in the epidemiology of blackleg caused by Pba by serving as an entry point into the roots and allowing the bacteria to move deeper into the roots leading to systemic infection. Both experiments above require repeating to confirm the results. The results of these and further experiments could determine whether the application of nematicides to commercial crops could play a role in reducing blackleg in potato. It could also link to work on irrigation to help in the development of a decision support tool for both seed and ware growers to help reduce blackleg incidence.

Next steps

The results from this work are being used to support an application for funding to the BBSCR, NERC, DERFA and Scottish Government research call on Bacterial Plant Diseases. The proposed project will further investigate the role FLN play in the epidemiology of potato blackleg caused by Pba.

Appendix 1

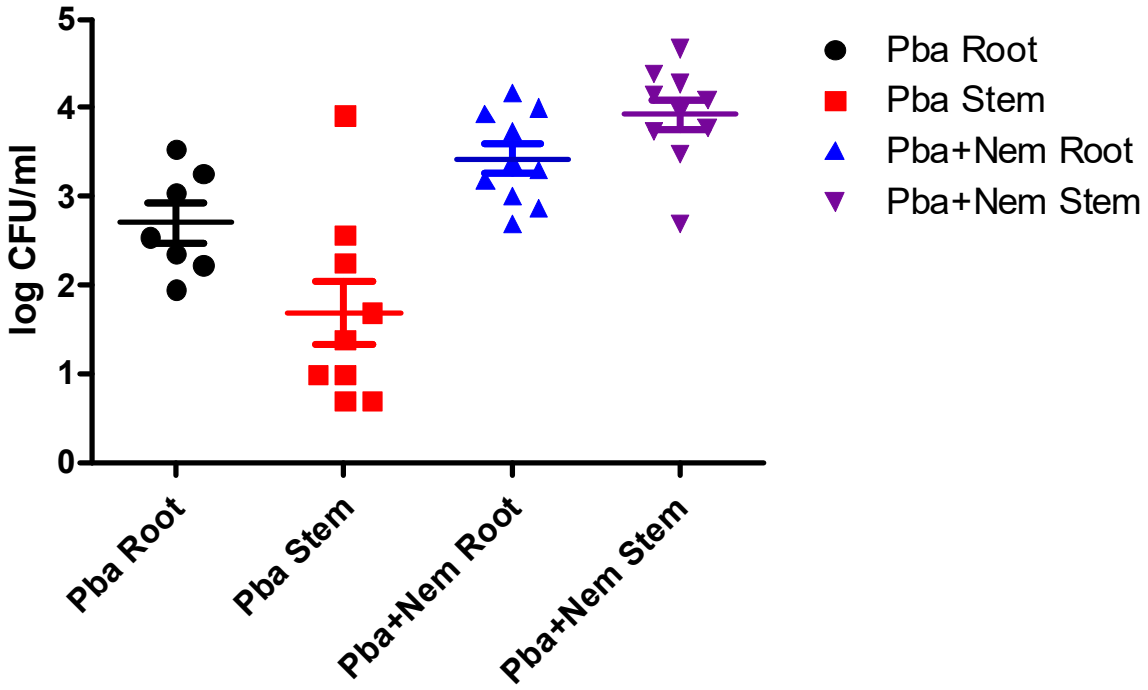
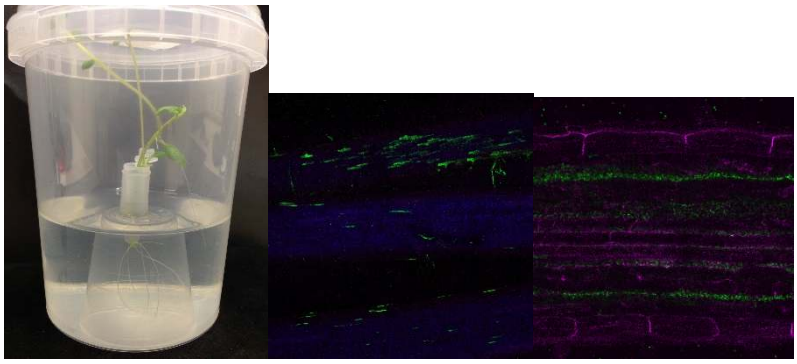


Figure 1. Graph showing effect of nematodes on *Pectobacterium atrosepticum* (Pba) internalisation of potato roots and stems. The results show a 10-fold increase of Pba in roots and a 100-fold increase in stems when nematodes are present.



A B C

Figure 2. (A) Micro-propagated plant in the hydroponic system; (B) Bacteria in the epidermal and cortex cells of roots after inoculation with *Pectobacterium atrosepticum* (Pba) only, and (C) Bacteria penetrating to deeper cell layers within the root and forming more extensive infections aligned longitudinally along root cell files after inoculation with Pba and nematodes.