Stacking of novel anti-nematode and anti-feeding site factors to obtain resistance against plant parasitic nematodes.

Project Co-ordinator: **John Jones (jjones@scri.sari.ac.uk)**
Research Partners: L. Castelli, C. Simpson, J. Brown, J. Jones.
Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA.
Industrial Partner: Advanced Technologies (Cambridge) Ltd, 210 Cambridge Science Park, Cambridge, CB4 0WA.

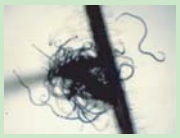
LINK COLLABORATIVE RESEARCH
SUSTAINABE ARABLE LINK PROGRAMME

ATC
Scottish Crop Research Institute

The problem: The potato cyst nematode (*Globodera pallida*).

Potato field infested with PCN to which two strips of nematicide have been applied.

- The potato cyst nematodes, *G. pallida* and *G. rostochiensis*, cause damage valued at over £50 million in the UK each year.
- The absence of natural resistance in commercially viable cultivars means that growers are forced to use potentially damaging nematicides, many of which are being phased out.
- Recent surveys have shown that *G. pallida* is spreading in the UK.

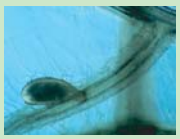


...or males that find and fertilise females forming...

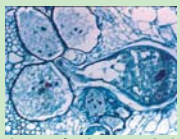
...cysts containing eggs each of which contains an ...

...invasive juvenile that hatches when a host (potato) is detected. After hatching....

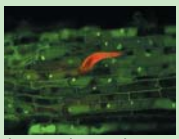
The PCN life cycle



...females that grow until they burst through the root...

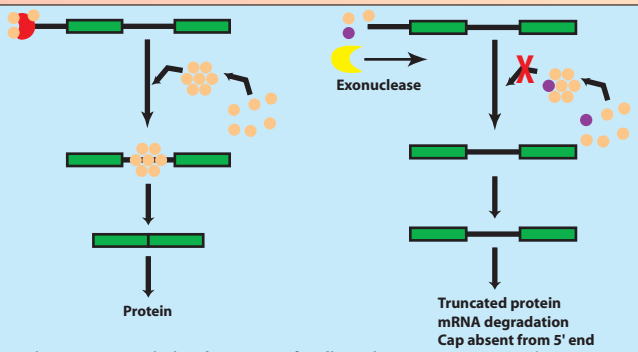


...and set up feeding sites (below). The nematodes then develop into...



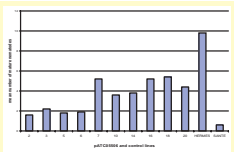
...the juveniles invade roots....

Anti feeding site approaches



Targeting post-transcriptional processes for disruption. Post-transcriptional processes require the assembly of multi-component enzymes which transcribe, translate and degrade the pre-mRNA. Disruption of these systems will inhibit splicing, disrupt capping and lead to mRNA degradation or production of truncated proteins.
Green boxes - exons; Lines - introns; Beige circles - Components of protein/RNA complexes; Purple circles indicate targeted components; Red - cap; Yellow - exonuclease enzyme..

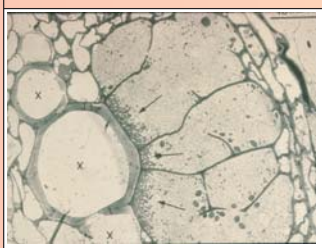
- Many pre-mRNAs add a guanosine cap to their 5' end. The cap has numerous roles in post-transcriptional processing including splicing, mRNA degradation, transport and translation. Disruption of capping is expected to lead to a rapid change in the numbers of functional mRNAs.
- The majority of plant genes are interrupted with non-coding sequence called introns, which are removed by a nuclear process called splicing using a ribonucleoprotein complex called the spliceosome. mRNAs are used in a number of rounds of translation before the message is degraded. Degradation is an active process that also involves assembly of multiple protein complexes on the mRNA leading to deadenylation and removal of the cap. The message is then open for exonucleolytic degradation. Disruption of this pathway is lethal.
- We targeted essential components of the spliceosome and linked them to a nematode responsive promoter. One target in particular had a significant effect on nematode development in transgenic potatoes. All selected transgenic lines of this target showed 50-80% fewer mature nematodes compared to the susceptible control potato variety, Hermes (see below). Analysis of these lines suggests that this



Nematode infection of transgenic lines expressing an inhibitor of splicing, susceptible (Hermes) and partially resistant (Sante) varieties. The mean number of mature nematodes detected in 10 randomised independent transgenic cell lines that contain inhibiting constructs are shown.

- This work provides the first example of targeting nuclear processes to disrupt pathogen attack and supports the principle of targeting such fundamental cellular processes.

The feeding site - a key adaptation and the nematodes achilles heel?

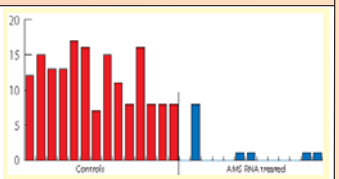


- After invasion the nematode induces a large, metabolically active syncytium (left) on which it feeds for the rest of its life.
- Once the syncytium is formed the nematode loses all body wall muscle and is unable to move. Disrupting the feeding site or nematode at this stage therefore inevitably leads to death of the nematode.
- Nematodes induce changes in plant gene expression in setting up syncytia.
- Genes activated in syncytia provide promoters that can give expression in the feeding site.
- We plan to link nematode responsive promoters to factors that will target the nematode or the feeding site and thus produce resistance. We have identified several candidate promoters for use with this strategy (see right).

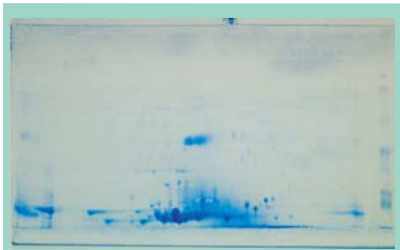
Expression of GUS (blue) at a feeding site from a nematode responsive promoter

Anti nematode strategy

- New techniques such as RNAi can be used to knock out selected genes in nematodes. The effects on the nematode can then be assessed (see right box).
- Essential nematode genes can therefore be selected for targeting.
- Nematode genes can be knocked out by delivering silencing factors (*e.g* double stranded or antisense RNA) to feeding nematodes at the syncytium.
- Anti-nematode and anti feeding site factors can be stacked, giving greater probability of control.



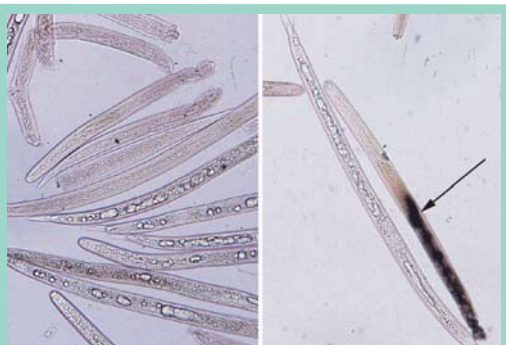
Results of infection experiments in which the ability of RNAi treated nematodes to infect is compared to that of controls. In this case treatment of nematodes with dsRNA from a gene expressed in sense organs has almost completely abolished the ability to infect plants.



2D gel showing proteins present in nematode body fluid - all the abundant spots at the base of the gel are derived from the lipid binding protein precursor

Target gene - lipid binding protein

- Single copy gene produces a polyprotein precursor - targeting a single mRNA should therefore ablate all mature peptides (see left).
- The gene is expressed specifically in the nematode digestive system (see right) - the first nematode tissue exposed to dsRNA from the feeding site.
- May transport lipids in nematode body.
- Disruption should be lethal - can be tested using RNAi (above)



in situ hybridisation reaction showing that expression of the PCN lipid binding protein is restricted to the digestive system. Nematodes incubated with a test probe show binding of the probe in the intestine, as indicated by a purple-brown stain (arrow). Control nematodes (left panel) show no binding of the probe.