Sustainability

Philip J. White

In the SCRI Annual Report 2009 we defined agricultural sustainability as the ability of a system to maintain stable levels of food production and quality in the long term, without compromising economic profitability or the environment. We described how SCRI scientists are developing agronomic practices and crop varieties that preserve soil fertility and reduce agrochemical inputs. Here, we consider the important role of soil monitoring in maintaining soil quality and report on several research projects promising novel strategies to minimise crop losses to pests and diseases.

The abundance and diversity of nematodes provides an excellent biological indicator of soil quality. Nematodes occupy key positions in soil food webs, and their populations respond rapidly to environmental perturbation. However, traditional methods for the characterisation of nematode populations are labour intensive and require trained taxonomists. To address this, Roy Neilson and colleagues have developed a simple, high-throughput, molecular method for characterising nematode assemblages that will allow the routine monitoring of nematode populations in soils. In addition to providing insight to soil quality, and the need for soil amelioration, this assay delivers information on nematode biodiversity, pest issues and bio-control opportunities.

About one third of potential food production is lost to pests and diseases. Historically, these losses were minimised by application of agrochemicals, but concerns over human and ecological wellbeing have restricted the use of these compounds. It is now imperative that crop varieties resistant to pest and pathogens are developed.

A common problem in developing crops resistant to pests and pathogens is the continued evolution of these organisms to circumvent the plant's defences. To develop durable resistance to the oomycete *Phytophthora infestans*, the organism that causes late blight in potato, Ingo Hein and colleagues are adopting a variety of approaches to identify, or create, plant defence systems that are initiated by molecules

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essential to the pathogen that are conserved during its evolution. Thus, a resistant plant will always recognise and respond to the pathogen. The genes underpinning this resistance might be used in marker-assisted breeding or deployed using GM technology.

Pavel Kerchev and colleagues are investigating the changes in plant gene expression that occur in response to the peach–potato aphid *Myzus persicae*, which is a major vector for *Potato leaf roll virus* in Scotland. They believe that an understanding of a plant's local and systemic responses to aphid infestation will provide useful information to develop technologies to disrupt aphid feeding or virus transmission, thereby reducing our reliance on synthetic insecticides.

The nucleolus of plant cells is intimately linked with the infection cycle of many viruses. Specifically, interactions with the nucleolus appear to be necessary for the movement of viruses to upper leaves. Michael Taliansky and colleagues seek to exploit their knowledge of how and why particular viruses target the nucleolus to develop novel, host-based strategies to control plant viral infections. One potential target is *Potato mop-top virus*, which has been responsible for reducing marketable yields of UK potatoes since 1966, and for which there is no known genetic source of resistance.

These examples of basic research providing intelligent, durable solutions to crop protection from plant pests and diseases reflect the necessity to avoid yield losses whilst reducing agrochemical inputs. Combined with innovative agronomic practices that preserve soil quality, and the development of crop varieties that make the best use of water and fertilisers, innate plant protection will enable the reality of sustainable agricultural production.

Molecular methods for monitoring soil nematodes and their potential use as biological indicators of soil health

Roy Neilson, Suzanne Donn[2], Xiaoyun Chen[1], Bryan S. Griffiths[1], Vincent O'Flaherty[3], & Tim J. Daniell

"*A nation who destroys its soil, destroys itself*" – Franklin Roosevelt, 1937 in a Letter to all State Governors on a Uniform Soil Conservation Law.

The Millennium Ecosystem Assessment considered soil as one of the world's most precious natural resources. Immediate threats to soils include erosion, compaction, change in biodiversity, contamination and loss of organic matter. Many of these threats can occur as a result of agronomic intensification to provide food for a burgeoning global population. Unsurprisingly, therefore, numerous recent Scottish and UK policy reports have recommended the maintenance and protection of soil. Monitoring is key to preserving soil quality, although tools for this are currently underdeveloped. At present, the lack of suitable indicators of soil quality makes it difficult to monitor the efficacy of processes employed to protect soil. Recently a Defra study assessed 183 candidate biological indicators of soil quality, of which 21 were ranked as having potential for deployment. Of those, the characterisation of nematode assemblages was viewed favourably, although limitations of throughput were identified as being a barrier to deployment.

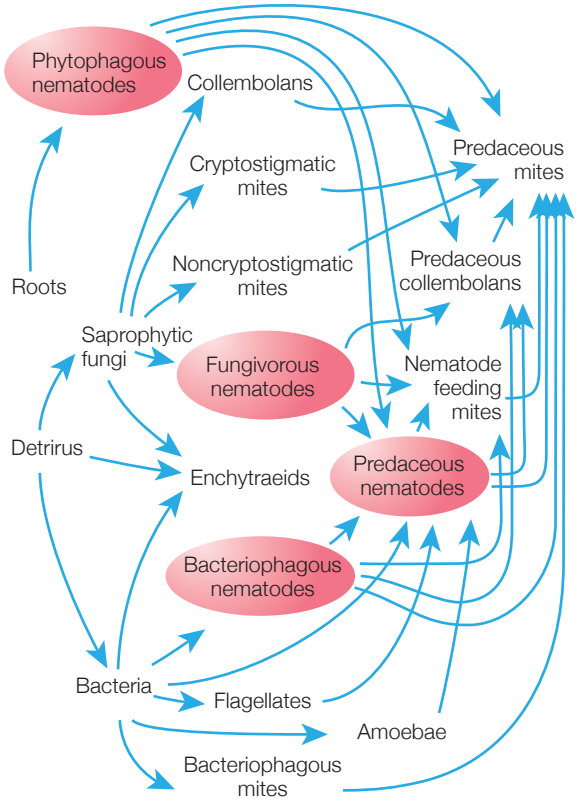

Figure 1 A schematic food web demonstrating the key positions of components of the soil nematode assemblages (after de Ruiter *et al.* 1993, *Journal of Applied Ecology* **30**, 95–106).

This view is supported by the position of nematodes at key nodes in soil food webs (Fig. 1), their contributions to soil functioning, high diversity and rapid response to changes in environmental conditions. There is good evidence that nematode phenotype ("biodiversity") is linked to function, with mouth parts commonly being used to identify feeding groups. Knowledge of species and assemblage structure can provide useful information on soil nutrient status and biodiversity as well as pest issues and bio-control opportunities, which are particularly important in ensuring sustainable food production. However, the time taken to identify a representative number of individuals from a soil sample presents a problem in the characterisation of nematode assemblages. This issue is compounded by the constantly declining number of skilled taxonomists.

Molecular tools provide an opportunity to address this problem. Using BBSRC funding at SCRI, we have designed a Directed Terminal Restriction Fragment Length Polymorphism (DT–RFLP) method that provides a high-throughput semi-quantitative characterisation of nematode assemblages. The DT–RFLP method is based on amplification of DNA with fluorescent primers followed by a diagnostic digestion with restriction enzymes to produce labelled Terminal–Restriction Fragments (T–RFs) linked to target taxa (Fig. 2). A software package, DRAT, has been developed in collaboration with the University of Dundee to identify appropriate restriction enzyme(s) to cut nematode DNA to yield diagnostic fragments. We have demonstrated that this method aligns well with classical taxonomic analysis when adjusted for nematode size (Fig. 3) and that it is applicable to soils from a range of habitats. A recent review of molecular tools for nematode monitoring concluded that DT–RFLP has advantages of high-throughput, ease of comparison between samples, and rapid data analysis. Furthermore,

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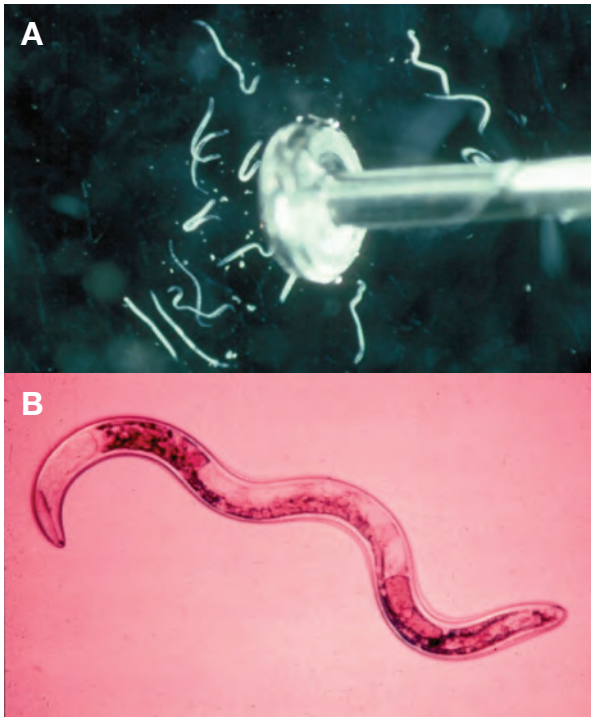

Figure 2 (a). A typical nematode assemblage isolated from arable soil illustrating the size and apparent uniformity under low magnification. (b) An individual *Trichodorus primitivus,* a free living nematode traditionally identified by high magnification by light microscopy, a requirement that restricts sample throughput. (c) An electropherogram of a DT–RFLP output illustrating discrimination of nematode assemblages below the taxonomic level of order allowing the identification of feeding groups (BF bacterial feeder, FF fungal feeder, OM omnivore, PF plant feeder and PR predator).

this technique allows comparison of samples from disparate geographic sites by converting the T–RF data into a univariate index similar to indices derived from classical morphological data.

To ensure applicability across the range of UK and other ecosystems, extensive trialling is required to develop a robust statistical protocol for analysing nematode

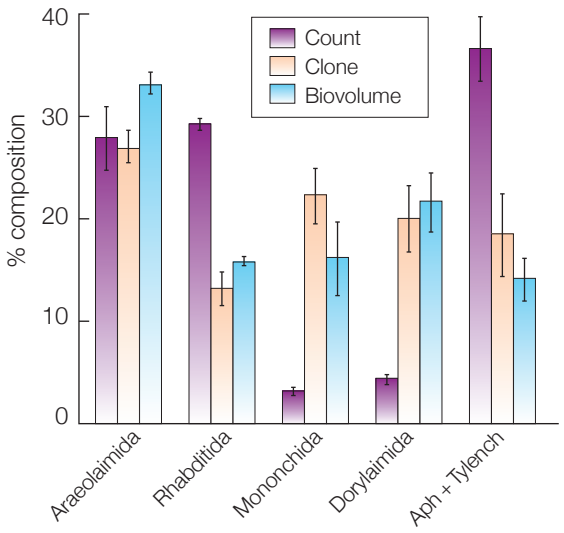

Figure 3 Characterisation of a single nematode assemblage at a taxonomic order level by cloning and sequencing (clone), morphological identification (count) and an assessment of biomass derived from counts (biovolume) (Aph – Aphelenchida, Tylench – Tylenchida); error bars represent standard error.

T–RF data. Furthermore, since nematodes are eutelic organisms (i.e. an individual of a species has a fixed number of cells, excluding epidermis and gametes), we are testing whether there is a correlation between group biomass and molecular signal in a joint project between SCRI, Teagasc and the National University of Ireland.

Pathogen effector driven search for more durable potato late blight resistance

Ingo Hein, Paul R.J. Birch, Sean N. Chapman & Glenn J. Bryan

Potato is the third most important food crop worldwide and makes a significant contribution to the UK bio-economy. The most significant threat to potato production is late blight disease, caused by the oomycete pathogen *Phytophthora infestans* that was responsible for the Irish famine in 1845–1846. Resistances to late blight have been introgressed from wild potato species but, despite intensive efforts, truly durable control mechanisms have remained elusive. The main challenge is to identify resistances that remain effective in response to changing pathogen populations.

Inducible plant resistances are based on pathogen recognition. All microbes trigger immune responses in plants via host receptor-mediated recognition of conserved pathogen-associated molecular patterns. However, successful pathogens suppress or otherwise manipulate this recognition via secretion of virulence factors called effectors. Effectors provide the plant with additional targets for recognition. In a second layer of inducible defence, resistance (*R*) genes, the products of which typically encode Receptor-Nucleotide Binding–Leucine-Rich Repeat (NB-LRR) proteins, detect effectors which are then termed avirulence proteins (AVRs). The key towards more durable resistance is to tailor resistances that target essential and conserved pathogen molecules that are more difficult to modify without loss of virulence. Such molecules present an 'Achilles heel' in the pathogen armoury.

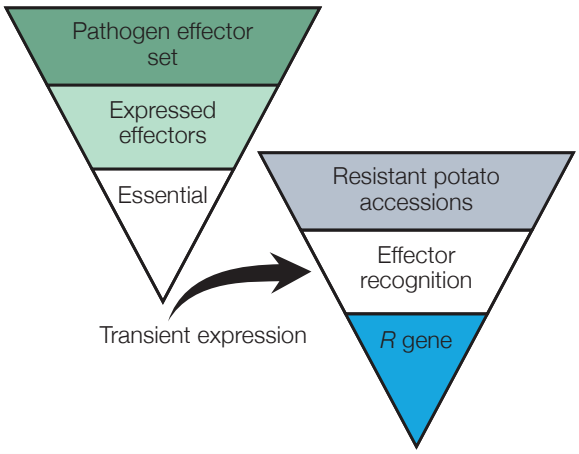

Figure 4 Pathogen effector studies are key to the identification of potentially more durable host resistance genes.

A number of *P. infestans Avr* genes have been identified. Although each corresponding AVR protein is distinct at the primary sequence level, they all share a signal peptide for secretion, followed by the motif RXLR which is required for translocation. More than 500 such candidate genes have been identified within the genome of *P. infestans*. The large numbers of RXLR genes raise the likelihood of functional redundancy in the effector complement. The implication of this is that, in addition to evolving critical amino acid mutations to evade recognition, effectors may also simply be shed if their function is provided by another effector.

In a 'paradigm-shift' from conventional disease resistance breeding we are exploiting our knowledge of recent dramatic changes in the pathogen population to unravel the underlying variation in pathogen effectors,

using this to drive the search for durable resistance. We seek essential and conserved *P. infestans* effectors as targets for cognate *Resistance to P. infestans* (*Rpi*) genes. In a two-pronged approach we seek *Rpi* genes that target all known allelic variants from these essential effectors.

The first approach (Fig. 4) utilises the diversity of *Rpi* genes within the Commonwealth Potato Collection (CPC) which contains more than 1800 potato accessions. Potato accessions that are resistant to diverse *P. infestans* isolates have been identified and are tested for their ability to recognise essential effectors. Genetic crosses are set up to demonstrate unequivocally the correlation between effector recognition and resistance. Furthermore, these segregating populations are used to characterise the resistance genetically and to isolate the underlying *Rpi* genes. A sequence analysis of the potato genome has predicted approximately 400 NB-LRR containing genes across all 12 chromosomes. We have successfully devised a strategy to enrich resistant and susceptible plants from segregating populations for *Rpi* genes and are using state-of-the-art next generation sequencing technology to identify more rapidly candidate *Rpi* genes and associated genetic markers. Effective *Rpi* genes can be deployed by marker assisted breeding or, more rapidly, via the use of GM technology.

The second approach utilises gene shuffling to alter the recognition specificity of cloned *Rpi* genes *in vitro*. Promising results have been obtained for the *Rpi* gene *R3a* that specifically recognises one form of Avr3a (KI) but fails to detect a virulent allele that differs from the recognised form in only two amino acids.

Using pathogen effectors as a driver to identify more durable resistance is a novel and generic approach that is amenable to every host–pathogen system. What is needed is knowledge of 1) the key effectors that are required for virulence, and 2) their diversity within pathogen populations. Resistances targeted to such effectors may be predicted to be more durable than those previously deployed and, by their nature, are intended to meet the challenge of future pathogen population changes.

Understanding local and systemic responses to aphid infestation as a tool towards breeding aphid resistant crops

Pavel I. Kerchev, Vandana Saraswat[1], Peter E. Hedley, Jenny Morris, Brian Fenton, Christine H. Foyer[2] & Robert D. Hancock

Plant feeding insects represent a significant problem in global agriculture, causing yield reductions and significant control costs. Worldwide, approximately 10,000 different insect species cause annual production losses amounting to £250 billion. One of the major groups of crop pests is the phloem-feeding aphids that can inflict serious damage through the removal of photoassimilates and by acting as vectors for plant viruses. For example, the peach–potato aphid (*Myzus persicae*) can colonise over 50 families of plant species, including a wide range of important crop plants, and can vector over 100 different plant viruses. In Scotland *M. persicae* is the major vector for *Potato leafroll virus*, a significant problem within the seed potato sector.

Figure 5 Confinement of aphids to a single *Arabidopsis* leaf within a clip cage.

At present aphid numbers are controlled primarily through chemical means and while pesticide application has boosted crop production in the short term, the energy inputs required to manufacture and apply pesticides, alongside their unintended negative impacts on beneficial insects, raise questions regarding the long term sustainability of pesticide use. Within this context, understanding plant defences against aphid infestation could lead to the development of novel technologies

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that disrupt aphid feeding and/or virus transmission. The mounting of defence responses against insects can be highly energetically and metabolically demanding for the host plant. Plants have therefore developed mechanisms to recognise insect infestation and only mount defence responses when necessary. These responses can be categorised into local, within the specific tissue being attacked, and systemic, that prepare the uninfested parts of the plant for subsequent attack. As aphids can feed from a single site for extended periods, systemic responses represent a significant, yet poorly studied, source of resistance.

As a first step to understanding systemic responses to aphid infestation, we examined global changes in gene expression in local and systemic tissues of the model plant *Arabidopsis thaliana* following infestation by *M. persicae*. Aphids were confined to a single leaf with clip cages (Fig. 5) and, following 6, 24 or 48 h exposure, infested and uninfested leaves from the same plant were harvested and their global gene expression compared with that of leaves from uninfested plants. Patterns of gene expression in local and systemic tissues were found to be highly divergent, suggesting very different strategies for defence in infested and uninfested systemic tissues. Further experiments showed that the changes in gene expression in systemic tissues were effective in reducing aphid survival. Plants were 'primed' by caging aphids onto a single leaf for 24 h. Subsequently, the aphids were removed and then one day old aphid nymphs were either caged onto the previously infested leaf or caged on to a separate uninfested leaf of a previously infested plant. Three days later, aphid survival was recorded (Fig. 6), indicating

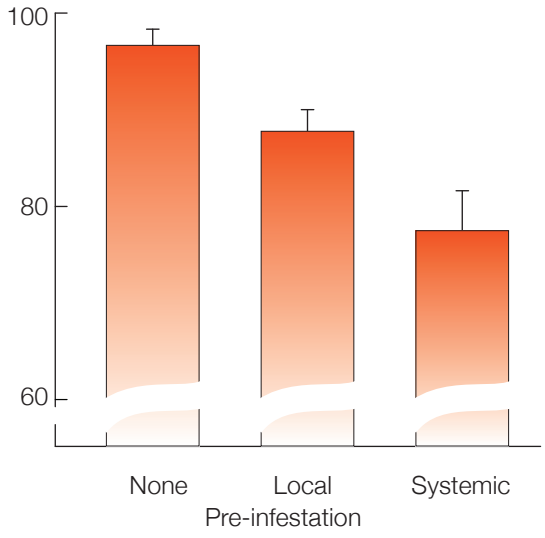

Figure 6 Impact of preinfestation on subsequent aphid survival on local and systemic leaves.

significant reduction in survival of those aphids caged onto previously infested leaves compared to those on control plants. Interestingly, survival on uninfested systemic leaves from previously infested plants was even lower, suggesting that the observed changes in gene expression result in an effective defence response.

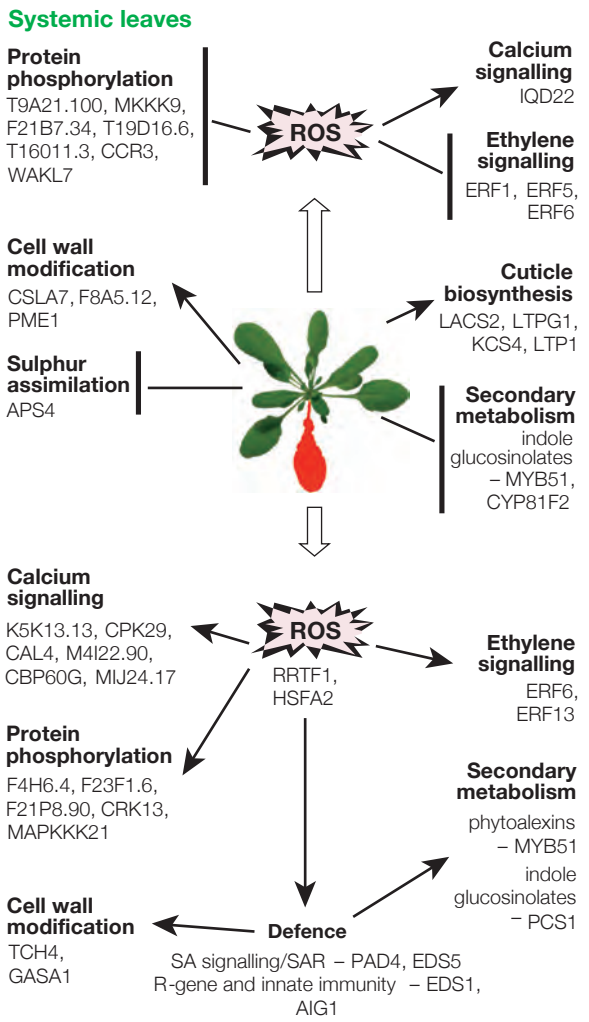

Figure 7 Overview of local and systemic signalling and defence following aphid infestation
The figure outlines signalling and defence cascades inferred from gene expression changes in local and systemic tissues following 6 h of aphid infestation on a single leaf. Signalling and defence cascades are represented in bold type. Upregulated processes are represented by arrows while downregulated processes are represented by blunt ends. Proteins encoded by differentially expressed genes are listed underneath the appropriate process. ROS – reactive oxygen species.

Analysis of gene expression changes occurring in local and systemic leaves allowed modelling of signalling mechanisms involved in recognition of aphid infestation and the pathways of defence (Fig. 7). This suggests a key role for the generation of reactive oxygen species in the local response, as has been proposed for microbial plant pathogens. Downstream signalling cascades include protein phosphorylation and dephosphorylation, via kinases and phosphatases, calcium signalling, and a role for the phytohormone ethylene. Defence appears to be mediated via cell wall modification, enhancing the physical barrier to aphid feeding, and through biosynthesis of toxic secondary metabolites. In systemic leaves, there was also evidence of heightened physical defence, through cell wall modification and enhanced cuticle biosynthesis. In addition, sulphur assimilation was reduced and the expression of a number of transporters was down-regulated, suggesting that plants may alter phloem composition to reduce nutritional value to aphids.

The work presented here provides novel insights into plant defence against aphid infestation. It is intended that this will drive novel genetic targets for breeding aphid resistant crops, thereby reducing reliance on synthetic insecticides and increasing agricultural sustainability.

Role of the nucleolus in plant virus pathogenicity

Michael E. Taliansky, Jane Shaw & Lesley Torrance

The nucleolus is a dynamic sub-nuclear body with roles in ribosome subunit biogenesis, mediation of cell stress responses, and regulation of cell growth. The nucleolus has also been shown to play a crucial role in the infection cycle of various viruses, and the nucleolar localisation of viral proteins has recently been described as a pan-virus phenomenon. In this regard, plant viruses are not different from other eukaryotic viruses. The past few years have brought remarkable progress in our understanding of how and why some plant viruses (in particular, umbraviruses and potyviruses) target the nucleolus and the functional role of the interaction between viral and nucleolar proteins in the

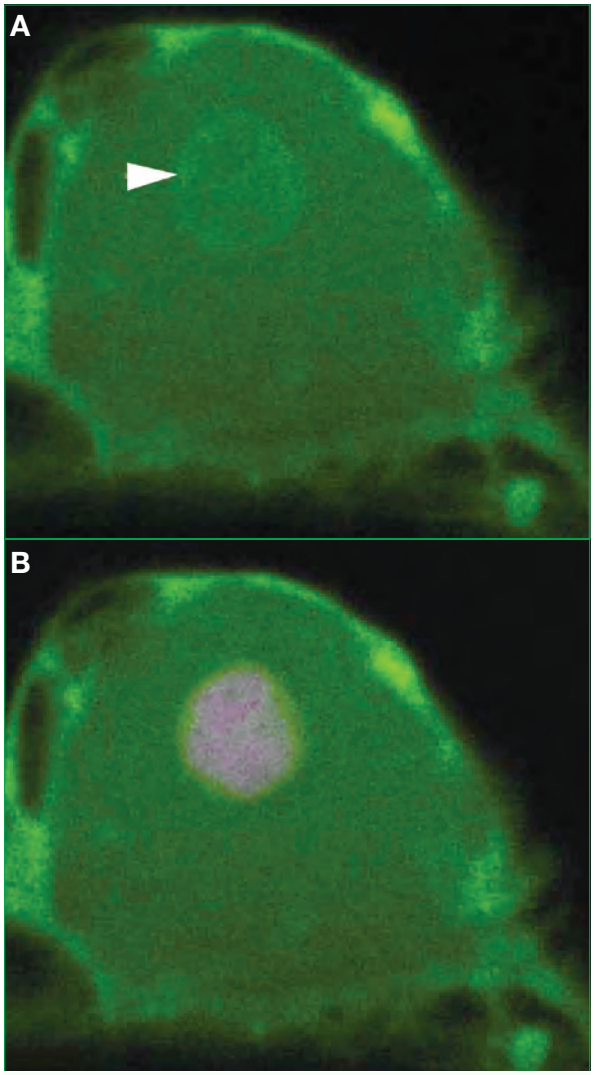

Figure 8 (a) Confocal microscope images of a nucleus showing the accumulation of PMTV TGB1 fused to green fluorescent protein in the nucleoplasm and nucleolus (arrowed). (b) The nucleolus is distinguished by the accumulation of fibrillarin labelled with red fluorescent protein (Wright *et al*. 2010, *Molecular Plant–Microbe Interactions* 23,1486-1497)

plant virus infection cycle. For example, interaction with the nucleolar protein fibrillarin is required for the systemic infection of plants by umbraviruses. Another example is *Potato mop-top virus* (PMTV) which affects potato tuber quality causing severe internal and external blemishes and yield loss in sensitive cultivars. We have recently shown that of one of the PMTV movement proteins (TGB1) associates with the nucleolus and this association may be required for viral movement to upper leaves (Fig. 8). Current experiments are focused on identifying the interacting components to understand fully their role in the virus infection cycle.

There are now several examples in which the plant viruses target other sub-nuclear bodies, associated with the nucleolus, such as Cajal bodies (CBs). In particular, for umbraviruses the role of CBs in nucleolar trafficking of the viral ORF3 protein has been established. The potential role of sub-nuclear structures in other plant virus infections is currently being addressed (Fig. 9).

The study of viral interactions with the nucleolus also provides unique and valuable tools to gain new insights into novel nucleolar functions and processes. For example, as previously discussed, the major nucleolar protein fibrillarin is involved in the formation and long-distance movement of umbraviral ribonucleoprotein particles, suggesting new unexpected functions for fibrillarin. We anticipate that more information will emerge about the mechanisms involved in regulating nucleolar function and structure in response to plant virus infections and hijacking the nucleolar functions for needs of the virus infection cycle.

Figure 9 Symptoms of *Turnip vein clearing virus* are strongly attenuated in *Nicotiana benthamiana* plants deficient in Cajal bodies (bottom row) compared with wild type plants (top row).

This fundamental work can be translated into practical use since, by identifying and understanding the components of the interactions (proteins and nucleic acids), both the plant cell and viral biology of the nucleolus can be exploited to design novel host factor-derived resistance strategies to control plant virus infections. This could provide a valuable alternative to control viruses such as PMTV for which there is no identified source of resistance in germplasm collections.

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