Detection of phytoplasmas in UK plants and insects

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Figure 1. The image shows a *Euleimonios montanus* insect of the subfamily Deltocephalinae. From http://www.agric.nsw.gov.au/Hort/ascu/staff.htm (Accessed 21/4/09).

Introduction

Have you ever wondered how your Christmas poinsettia came to have its characteristic bushiness (see figure 2)?

Figure 2. A poinsettia plant. From . http://www.plantmanagementnetwork.org/pub/php/review/xmasflower/images/poinsettia1sm.jpg (Accessed 4/11/09)

Perhaps not…! The reason is that the plant is infected with phytoplasmas. Phytoplasmas are a type of bacteria which are capable of living inside both plants and insects. Unfortunately the aesthetically pleasing result of phytoplasma infection seen in a poinsettia plant is greatly overshadowed by the harmful effects in others.

Phytoplasmas have been found in many different species of plant across the world. One of the most dramatic examples of damage in a crop species is seen in the coconut plant, see figure 3. Similar economic losses occur for the growers of pears and celery (Smart *et al.*, 1996). Phytoplasmas also cause diseases in potatoes, including purple top wilt found in Europe and North America, where they cause problems for growers by decreasing quality and yield (Secor., 2008).

Phytoplasmas are small bacteria, of which there are several different groups and sub-groups. It has recently been accepted that the most likely method of infection is by insect transmission

Figure 3. Yellowing in coconut palm (USDA Forest Service - Region 8 Archive, USDA Forest Service, Bugwood.org) (Accessed 4/11/09).

Including leaf hoppers (Weintraub and Beanland, 2006), see figure 1.

The insects which are able to spread phytoplasma infections are best suited to environments warmer than the UK. Therefore there have only been a few reports of phytoplasmas in the UK to date (Jones and Baker, 2007), however, global warming has the potential to change this situation in the relatively near future. This makes the study of phytoplasmas and their vectors very important.

A selection of plants and insects from two sites in the UK were tested for phytoplasma infection. Alongside these experiments the methods employed were tested for accuracy and

suitability, if the methods could be improved this would help with the diagnosis of disease in the future.

Materials and Methods

Fifteen samples were taken from plants at the Sutton Bonington campus of the University of Nottingham in Leicestershire, see table 1.

Name of plant sample
Grass 1
Grass 2
Grass 3
Petunia 1
Petunia 2
Petunia 3
Tomato
Wild Carrot
Day-lily
Chinese Lantern
Apple Tree
Unidentified Tree
Green Beans
Single Leaf Ash
Unidentified Bush

Table 1. Samples from plants at the Sutton Bonington campus, Leicestershire, UK. Those samples in italics were collected from the glasshouses. The remainder were randomly sampled from around the campus.

To assess phytoplasma contamination in a sample of UK insects, DNA was also extracted from insects collected from Kenfig Sands, Newport, South Wales, which were provided by Mike Wilson of the National Museum of Wales.

To assess the method, a further set of insects was sent from The John Innes Institute, Norwich by Saskia Hogenhout. These insects had been raised on plant material infected with two different strains of phytoplasma. These were used as controls in the methods testing.

The DNA of the plants was extracted and amplified using the polymerase chain reaction (PCR). The extracted and amplified DNA was cut into sequences using enzymes, a technique known as restriction fragment length polymorphism (RFLP). The sequences, which have different molecular weights, were characterised by separation on an agarose gel by electrophoresis. The gel acts as a molecular sieve separating the DNA by mass.

The DNA sequences of the insects and plants used in the study were available in a bioinformatics database. A computer program called pDRAW32 was used to create virtual images of the agarose gels which could be compared with those following RFLP agarose gel electrophoresis, revealing the changes due to phytoplasma infection.

Results

Of the fifteen plant samples tested (see table 1) three were consistently positive for phytoplasma DNA, see figure 4. These were apple, day-lily and wild carrot. Comparison of their DNA sequences with that of phytoplasma DNA revealed that all three plants were infected with the same type of phytoplasma from the aster yellows group.

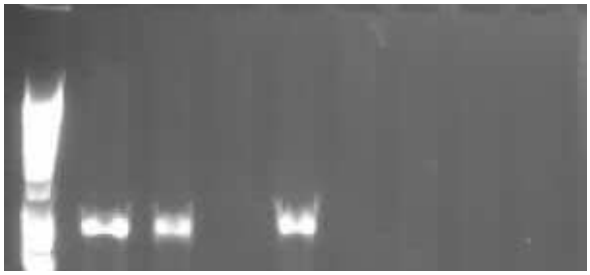

Figure 4. Agarose gel electrophoresis of plant samples. Far left is the ladder, which contains all possible bands. To the right are three bands , one for each of the three plants which were positive for phytoplasmas.

Of all the insects tested from South Wales only one was consistently positive. This was *Delphacidae muellerianlla,* a leaf hopper. However, subsequent analysis of sequenced DNA revealed that the insect was not infected with a phytoplasma but instead with a different type of bacteria called a *Wolbachia,* an endosymbiotic (lives within another organism) bacterium.

Figure 5 shows the results from two insects at The John Innes Institute which were infected with different phytoplasmas. The maize bushy stunt phytoplasma and aster yellows witches broom phytoplasma were found in *Dalbulus maidis* and *Macrosteles quadrileantus* (leaf hopper) respectively.

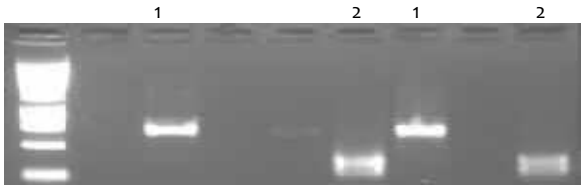

Figure 5. Agarose gel electrophoresis of the RFLP of two types of insect from The John Innes Institute. The four bands are from insects positive for phytoplasma. Bands 1 are from *Dalbulus maidis* and bands 2 are from *Macrosteles quadrinleanus.*

The virtual gels of phytoplasma DNA were created using pDRAW32, see figure 6. Each manipulated image was for just one restriction enzyme, showing the banding patterns expected for different phytoplasmas. The restriction enzymes used included *Alu*I, *Hae*III, *Mbo*I, *Rsa*I and *Taq*I.

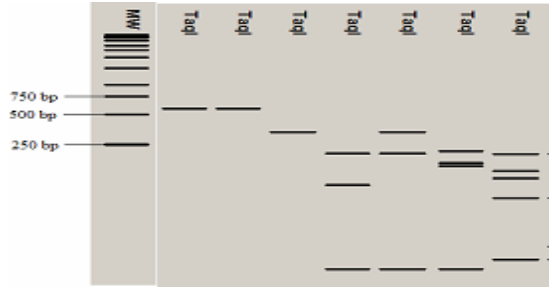

Figure 6. Virtual RFLP, using *Taq*I restriction enzyme. Seven different strains of phytoplasma were used to create the bands.

Discussion

This study suggests that some plants at Sutton Bonington are infected with phytoplasmas. Three of the fifteen plants tested were positive. All three were infected with aster yellows phytoplasma, a strain previously found in the UK (Jones and Arocha, 2006). Whilst this is encouraging for the reliability for the results of this study, it is sadly further confirmation that phytoplasmas are present in the UK, with the potential for spread and plant loss. Should the disease reach crop plants the results could be very serious. Aster yellows phytoplasma causes disease in many plants including onions, tomatoes and carrots. It can cause many problems including stunted growth and sterility. The leaf hopper *Macrosteles quadrinlineatus* is known to transmit the aster yellows phytoplasma. However, other methods such as seed transmission may also be possible (Weintraub and Beanland., 2006).

None of the insects from South Wales tested positive for phytoplasmas. This sample of insects can be used to suggest that the insects which carry phytoplasmas are not currently active in the area. The insects fed on infected plants from The John Innes Institute were positive for their strains of phytoplasma, providing useful information on the reliability of the method.

Temperatures across the UK, and the world are rising due to global warming, this will create environments which are more suitable for the insects, which spread phytoplasmas. Although no insects were found to be infected in this study, samples were from only one location in the UK. With changing temperatures the outcome may be very different in the future. In addition, the trend towards organic farming and the reduction in pesticide use will allow the insects to survive, therefore increasing the potential for the spread of disease (Hogenhout *et al.*, 2008)

The use of pDRAW32 to create the virtual RFLP images provides a valuable tool for future diagnosis of phytoplasma infections. A sample can be collected, put through PCR, RFLP and then agarose gel electrophoresis. The result would be compared to a virtual gel (having used the same restriction enzyme) to see if the banding patterns match. This would give a quick, preliminary idea of which, if any phytoplasmas may be present in the sample.

Future studies

Any future study would aim to test more plants and insects from across the UK to consider the extent of phytoplasma disease in the UK.

There is some information which suggests that the ability of an insect to carry phytoplasmas depends on its age and sex. Experiments using a range of ages and sexes would expand the knowledge in this area.

Further reading

Hogenhout, S.A., Oshima, K., Ammar, E., Kakizawa, S., Kingdom, H.N. and Namba, S. (2008) Phytoplasmas: bacteria that manipulate plants and insects. *Mol. Plant Pathol.* 9, 403-423.

Jones, P. and Arocha, Y. (2006) A natural infection of Hebe is associated with an isolate of '*Candidatus* Phytoplasma asteris' causing a yellowing and little-leaf disease in the UK. *Plant Pathol.* 55, 821-910.

Jones, D.R. and Baker, R.H.A. (2007) Introductions of non-native plant pathogens into Great Britain, 1970–2004. *Plant Pathol.* 56, 891-910.

Secor, G.A. (2008) The Canon of Potato Science. *EAPR*. 50, 255-257.

Smart, C.D., Schneider, B., Blomquist, C.L., Guerra, L.J., Harrison, N.A., Ahrens, U., Lorenz, K.H., Seemuller, E. and Kirkpatrick, B.C. (1996) Phytoplasma-Specific PCR Primers Based on sequences of the 16S-23S rRNA Spacer Region. *Appl. Env. Microbiol.* 62, 2988–2993.

Weintraub, P.G. and Beanland, L. (2006) Insect Vectors of Phytoplasmas. *Annu. Rev. Entomol.* 51, 91-111.

Author profile

Laura is 22 years old and studied in the School of Biology, graduating in 2009 with a first class degree, BSc. Biology. Laura was especially interested in plant science, particularly plant disease. She is now pursuing a career in this field.