



# Psyllid News

May 2010

## Psyllid News - Special Edition

This issue of Psyllid News focuses on testing potato plants and tubers for *Liberibacter* and *Phytoplasma*. There have been a lot of questions about testing potato tubers or foliage to determine whether they are free from *Liberibacter* and *Phytoplasma*. Growers have also been asking where they should send their samples for testing. In this newsletter we try to answer these questions.

The next edition of Psyllid News will be published at the end of June, and will present results of this year's research projects and include a scan of international research of interest to growers.

If you have received this by post but would prefer to receive it by email, please let Kate ([kate.shannon@hortnz.co.nz](mailto:kate.shannon@hortnz.co.nz)) know your email address.

## Who provides a testing service?

Three laboratories offer services for testing potato material for *Liberibacter* and *Phytoplasma*: Plant & Food Research, Lincoln; MAF IDC-PHEL, Auckland; and Linnaeus Ltd, Gisborne. All three laboratories offer a high quality of service. The MAF IDC-PHEL will test material if no other commercial service is available; their primary role is diagnostic work for pest and disease incursions ([www.biosecurity.govt.nz/pests/plants/pHEL](http://www.biosecurity.govt.nz/pests/plants/pHEL)). Linnaeus is a commercial laboratory that is about to launch a commercial testing service for *Liberibacter* and *Phytoplasma* ([www.linnaeus.co.nz](http://www.linnaeus.co.nz)). Plant & Food Research ([www.plantandfood.co.nz](http://www.plantandfood.co.nz)) will also provide commercial testing, but the main focus of their laboratory is psyllid research, including work to develop techniques for better detection of *Liberibacter* and *Phytoplasma* in potato samples.

## So which lab is best?

We have discussed this question with the three laboratories. Although they use slightly different testing methods we are confident that any differences between test results would be very minor and not impact on the decisions being made by growers.

Because *Liberibacter* and *Phytoplasma* are usually present at a very low concentration there will always be some variability between test results, regardless of where they are tested. If a laboratory tested 10 samples from one plant it may not get the same result for each test - this will happen regardless of which laboratory samples are sent to. For that reason it is best not to just send one plant for testing (see *Sampling* below).



Foliar symptoms of *Candidatus Phytoplasma australiense* in a Fianna potato. Image courtesy of Lia Loeffing, MAF IDC-PHEL.

## Interpreting test results

Testing reports may list two outcomes of the testing:

- Positive
- or
- Not detected.

A positive test result means that either *Liberibacter* or *Phytoplasma* (as specified in the report) has been detected and is present at some level in the crop. It is currently unclear whether *Phytoplasma* causes any damage to potato crops or tubers, but research into this question should be completed in late 2010. This means that a positive *Phytoplasma* result should be interpreted with caution. *Liberibacter* on the other hand is known to have a serious impact on crop development and crops testing positive for *Liberibacter* will probably go on to develop zebra chip as well as foliage yellowing and dying. *Liberibacter* is known to be seed transmitted and infected seed can produce infected plants. However the precise role of *Liberibacter* on seed aging, germination, and spread of disease in subsequent crops is still unclear (refer to the April issue of *Psyllid News*). A further update on this will be provided in the June issue of *Psyllid News*.

A "not detected" result is even more difficult to interpret. It may mean that *Liberibacter* or *Phytoplasma* are not present in the crop or tubers. But equally it may mean that one or both are present, but at levels too low to detect. That is why none of the laboratories

will report results as "negative" or "absent". For example an infection may be at a very early stage and unable to be detected. As yet we don't know how important these very low levels of *Liberibacter* and *Phytoplasma* are.

## Sampling

When deciding how big a sample to submit for testing, or whether to test at all, the first thing you should ask yourself is "why do I need this information, and what decisions am I going to make based on the results?"

The best sample is a very large one as this will maximise the chance that *Liberibacter* or *Phytoplasma* will be detected if they are present - but it is also very costly as the laboratory will have to test each plant or tuber separately. Unlike virus testing, where virus is present at a high level in the plant, the amount of *Liberibacter* and *Phytoplasma* may be so small that bulking samples together could dilute the bacteria to a level where they are non-detectable.

Foliage samples should be a whole plant which shows symptoms of *Liberibacter* or *Phytoplasma* (these symptoms are quite similar – see [http://www.potatoesnz.co.nz/user\\_files/PDF/Psyllid\\_fact\\_sheets\\_-\\_complex\\_lr.pdf](http://www.potatoesnz.co.nz/user_files/PDF/Psyllid_fact_sheets_-_complex_lr.pdf)).

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Zebra chip symptoms in a freshly dug potato.  
Image courtesy of Stephen Ogden, Market Access Solutionz.



Foliar symptoms of *Candidatus Liberibacter solanacearum* in a Moonlight potato. Image courtesy of Lia Liefting, MAF IDC-PHEL.

## What is PCR?

PCR stands for Polymerase Chain Reaction, which is a technique for multiplying the amount of DNA in a sample up to a level where there is enough of it to be identified. The key term is "chain reaction". There's a lot of DNA in a plant sample, but only a tiny amount of Liberibacter or Phytoplasma DNA. The PCR amplifies the DNA that we want while leaving the amounts of other (plant tissue) DNA unchanged. It does this by using a set of primers that are specific to Liberibacter or Phytoplasma.

DNA is like a zipper - one half zips up with the other half. The teeth of the zipper are represented by four protein-like molecules (nucleotides) denoted C, T, A, and G. C only ever binds to G, and A only binds to T. The Liberibacter and Phytoplasma primers are pieces of DNA specific to these diseases, and which act like a shorter piece of the zipper.

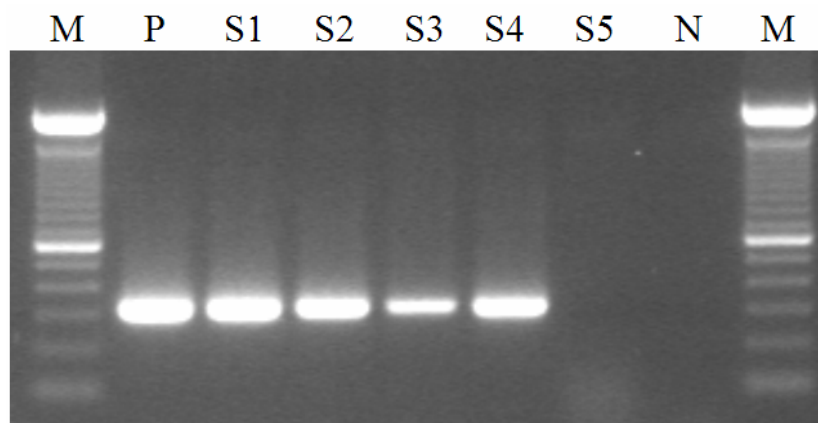
The PCR machine heats the DNA until it unzips. It then cools down and as it cools the primers bind onto the unzipped Liberibacter or Phytoplasma DNA, doubling the amount of target DNA in the sample. The PCR cycle continues until the target DNA has doubled in number by around 40 times (this multiplies one strand of target DNA into 1,099,511,627,776 strands).

There are several identification methods which use the PCR technique. The main two methods are nested PCR and real-time PCR. Plant & Food Research are also investigating other PCR techniques in order to increase the sensitivity of detection of Liberibacter and Phytoplasma, and to reduce testing costs.

In nested PCR the sample tubes are then opened and another set of primers are added. The PCR is then run another 40 cycles. The advantage of nested PCR is that it is very sensitive, doubling the amount of target DNA eighty times. A disadvantage is that there is potential for cross contamination when opening the tubes to add the second set of primers. After the reaction is complete the PCR products are placed onto a gel through which an electrical current is passed. Molecules move through the gel with the electrical current in accordance with their size. Small molecules move quickly and larger DNA fragments move slowly. The target DNA spreads along the gel and forms bands on the gel. These are then stained with a DNA-binding dye which can be viewed under UV light. Positive samples will produce a band of the correct size when compared to the positive control.

The process is time consuming and costly, and for these reasons some commercial testing laboratories do not use nested PCR, instead opting for "real-time PCR".

Real-time PCR follows the same principles of using primers and heating and cooling in a PCR machine, but only one pair of primers is used in up to 40 cycles. Instead of running the products on gels for identification, a dye is added as part of the reaction mix. This dye (a fluorescent reporter probe) fluoresces when it binds to target DNA. This fluorescence is detected by the machine, indicating the presence of target DNA. The relative quantities of target DNA in different samples can be measured, which is why this method is sometimes called quantitative PCR.



PCR products after gel electrophoresis viewed under UV light. DNA extracted from 5 samples (S1 to S5) was tested by PCR. Positive (P) and negative (N) controls were included in the test. DNA size markers (M) were included on the gel to estimate the sizes of the PCR products. Samples S1-S4 tested positive. Image courtesy of Lia Lieffing, MAF IDC-PHEL.

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Random sampling should be avoided as disease distribution in the crop tends to be patchy. Targeting symptomatic plants will maximise the chances of positive test results.

Testing seed tubers will require a large sample to be submitted if a meaningful result is to be obtained. Unless the crop has been badly affected with *Liberibacter* or *Phytoplasma* then the proportion of infected tubers will be quite small. Testing of tubers can also produce variable results, so if you submit ten randomly selected tubers from a seed line the chances of a positive result are very low even if *Liberibacter* or *Phytoplasma* are present in the crop - if the result is "not detected" how would you interpret the results?

If you have reason to be concerned that a seed line may have *Liberibacter* it may be possible to pre-screen the samples before submission. Look for the distinctive "pink belly

button" – the pink, wrinkled and collapsed area of the tuber where the stolon was attached (see photos below). In older seed look for tubers that are shrivelled, or which are prematurely sprouting. Cutting these suspect tubers may reveal zebra chip symptoms, which can then be confirmed by laboratory testing.

### Summary

We advise you to think carefully about how you will interpret the results of any testing before investing in this. Bear in mind that while a positive result tells you something, "not-detected" may not mean the crop is free of the disease. Talk to the laboratory about sample size, sample collection, turn-around time, costs, and interpretation of results before collecting the samples. We have confidence that all three laboratories will provide a high level of accuracy in their test results. How you interpret the results is more important than your choice of laboratory.



The distinctive "pink belly button" symptom of *Liberibacter* infection. Images courtesy of Stephen Ogden, Market Access Solutionz.

Front page header image courtesy of Joseph E. Munyaneza, USDA

## Potatoes New Zealand

[www.potatoesnz.co.nz](http://www.potatoesnz.co.nz)

Ron Gall (Business Manager)  
Horticulture New Zealand  
PO Box 10 232  
Wellington

Phone 04 472 3795  
Fax 04 471 2861  
Mobile 027 446 6838  
Email [ron.gall@hortnz.co.nz](mailto:ron.gall@hortnz.co.nz)

Stephen Ogden (Psyllid Coordinator)  
Phone 04 473 6040  
Fax 04 473 6041  
Mobile 021 773 502  
Email [stephen@solutionz.co.nz](mailto:stephen@solutionz.co.nz)