Phytoplasmas, a new threat to New Zealand plants?

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In the late 1980s there was much concern in the community about dying cabbage trees. Since then, I have been involved with many colleagues from Landcare Research and HortResearch at the Mt Albert Research Centre studying what was going on. This article provides an update on progress and indicates some gaps that remain.

We named the cabbage tree disease sudden decline, reflecting the rapid death of affected trees (Rees-George et al. 1990). There has been much speculation as to the cause of the disease. However, answers require more than talk. We have made many observations, and carried out diverse experiments, investigating possible reasons. Here I summarise our findings. It is something of a detective story: a tale of mysterious deaths, of historical links, of the power of DNA techniques, of international connections, of unexpected findings, of some convictions, and of some unsolved mysteries.

To obtain some real measure of what was happening, we undertook repeated observations of the health of over 1700 individual cabbage trees beginning in late 1988 (Rees-George et al. 1990, Beever et al. 1996). In the northern half of the North Island (north of Taumaranui) about 11% of the trees under observation were lost each year, whereas less than 1% of trees in the southern North Island and northern half of the South Island died each year. This result indicated there was indeed a disease epidemic. Unusually large numbers of trees were dying in the northern North Island, far more than were being replaced through natural or assisted regeneration.

We have now traced the cause of the disease to a specialised bacterium, a phytoplasma (Fig 1).

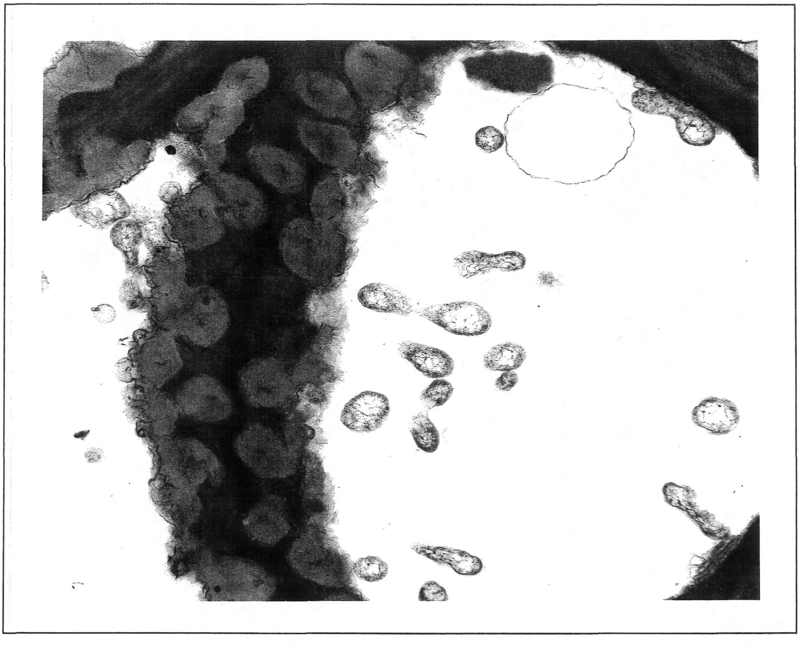

Fig 1. Transmission electron micrograph of the cabbage tree phytoplasma in the sieve tube cell of an affected tree. The veritable 'needle in the haystack'. The dark wall to the left side of the image is the sieve plate, with the sieve pores showing as circular grey structures. The circular and dumbell-shaped objects in the left and right compartments are phytoplasmas.

The evidence that this bacterium is the cause of sudden decline is diverse. It was submitted to the international journal 'Plant Disease' in the form of a scientific manuscript. As with all such journals, the manuscript was passed to other scientists expert in the field for review. These reviewers accepted the evidence and arguments we made, the manuscript was accepted for publication by the journal editors, and it is now in print (Andersen et al. 2001) .

While the electron micrograph is direct evidence the bacterium is present in some affected trees, this presence is not sufficient in itself to prove the bacterium is the cause of the disease, not least because we have looked at parts of other affected trees and not found phytoplasmas by this method. However, electron microscopy is a time-consuming and relatively insensitive method of detecting phytoplasmas. Thus we turned to other more sensitive detection methods, and in particular to techniques for detecting the DNA of phytoplasma bacteria in the plant. These 'DNA techniques' allow relatively large samples and numbers of tissue to be examined. Using these techniques, which are similar to those used in police forensic work, we were able to detect the phytoplasma in at least one part of all affected saplings and some affected adult trees examined, but not in any unaffected individuals. The most reliable tissue to examine turned out to be the apex of the rhizome, which in the case of adults makes sampling difficult because the apex can be a metre or more deep in the ground. Furthermore, the amount of phytoplasma DNA detected in adults was very low and only transiently present. In total, these findings allowed us to conclude that the phytoplasma is present in affected plants and not in unaffected ones. Such a correlation does not allow us to conclude that the phytoplasma is the cause of the disease, but rather that is regularly associated. Our conclusion that it is the cause comes from observations of the symptoms coupled with experiments using antibiotics. The symptoms, including leaf yellowing and rapid plant decline, resemble those of other phytoplasma diseases such as lethal yellowing of palms. Moreover, they indicate the infection begins in one of the leafy tufts and spreads down the plant to the rhizome and roots, a pattern consistent with a disease agent being spread by leaf-feeding insects. The antibiotic experiments indicate that injections with oxytetracycline, an antibiotic known to affect phytoplasmas, can 'cure' affected plants.

Phytoplasmas are specialised bacteria that were once called mycoplasma-like organisms or MLOs. They are unusual in that they lack a true cell wall, and are merely enclosed by the cell membrane. For this reason they may be variable in size and shape, sometimes appearing round in cross-section, sometimes elongate or even dumbell-shaped (Fig. 1). They inhabit the sugar-filled phloem sieve tubes in host plants and also live in the salivary glands of insects that transmit them. These insects feed by sucking up the phloem sap, thereby ingesting the phytoplasmas as well as their liquid food. Relatively little is known about which insects transmit phytoplasmas to which plants. Phytoplasmas are not seed transmitted, and cannot be transmitted plant to plant except by insects or by grafting. They are difficult to study because they cannot be grown in culture in the laboratory.

Only one species of phytoplasma is known in New Zealand, with the common name Phytoplasma australiense (Liefting et al. 1998). It is the cause of yellow leaf disease of flax (*Phormium tenax*), which was first recognised in 1908 and initially thought to be a physiological disorder. Subsequently, various researchers investigated the problem without establishing a cause. Research stopped during the Second World War but an intensive study of the disease, involving a team of researchers including the late Prof. Frank Newhook, long-time ABS member, was initiated in 1944 (Boyce & Newhook 1953). This work showed the disease was transmitted by the flax planthopper *Oliarus atkinsoni* (Cumber 1953). Although it was initially thought to be a virus, it was later shown to be a phytoplasma (Ushiyama et al. 1969). Leaves of affected plants turn abnormally yellow and brown off, and the whole clump eventually collapses. Yellow leaf is widespread in natural and semi-natural sites throughout much of the country. It was one of the causes of the eventual collapse of the now defunct flax fibre industry.

Phytoplasma australiense also causes a disease in strawberry, a very different host from flax or cabbage tree. Lethal yellows of strawberry (Andersen et al. 1998) is of particular concern to strawberry-runner growers in the Katikati region, who may lose up to 10% of the plants in some plots. Symptoms include a reddening of the leaf margin and a general unthriftinesss of the plants, leading to loss of runner production.

The distribution of affected strawberry plants suggested to us that the infective insects were being blown into the strawberry plots from an upwind source. In 1998 we sampled a range of unhealthy looking trees and shrubs upwind from an affected strawberry plot in Katikati. Using the DNA test, we found the phytoplasma in karamu (*Coprosma robusta*) showing leaf loss and tip dieback. We realised that similar looking plants were common around Auckland, and soon confirmed the presence of the phytoplasma in such plants. We named this disease lethal decline (Beever et al. 2000). It is not clear how long karamu has been affected by this disease but the late Katie Reynolds, in a letter written to me in 1985, commented that she wondered whether some dieback of taupata she had seen on Bream Island in the Whangarei harbour was afflicted by "that die-back thing that has come to the coprosmas in recent years". Interestingly, this would be about the time cabbage trees were beginning to die in large numbers. While leaf loss and dieback symptoms are common in karamu throughout the North Island and northern half of the South Island, we have yet to confirm the symptoms are always associated with the phytoplasma.

As well as affecting cabbage tree, flax, strawberry and karamu in New Zealand, P. australiense is also present in Australia. There, it is only known from two introduced species: grape and papaya. It causes an important disease of both: in the case of grape a slow decline disease called Australian grapevine yellows, and in papaya a rapidly lethal disease termed papaya dieback (Liefting et al. 1998).

So, what is happening? Our results so far suggest that the phytoplasma is native to flax in New Zealand, and in the last half-century or so has moved out from flax to affect other plants. It may have been transported to Australia in affected flax rhizomes before effective quarantine was established. More knowledge of the insects that transmit the phytoplasma is needed to confirm this hypothesis. To date, the only insect known to transmit P. australiense either in Australia or New Zealand is the flax planthopper, a species largely restricted to its flax host. Thus we presume other species are involved, and while we suspect the passionvine hopper and the green planthopper, experiments to date have failed to implicate either.

One clue as to how the disease is spreading may come from studies of the variation in the phytoplasma population. Are there strains specific to each host, that is a flax strain, a cabbage tree strain, etcetera? Or is the phytoplasma moved from one host to another, for example, from karamu to strawberry? We hope to answer this question by studying the variation in DNA strains from the different hosts. We strongly suspect that cabbage tree is a terminal host, that is, the phytoplasma does not move from one cabbage tree to another but rather is transmitted into cabbage tree from karamu for example. Similarly, we suspect strawberry are also infected from karamu.

The diverse species affected by P. australiense, and the subtlety of symptoms it produces in some species such as karamu, suggest other species may also be affected. We suspect that puriri (*Vitex lucens*), which often shows tip dieback, may be affected, as may the mamaku (*Cyathea medullaris*). However, the limited work we have done on these species to date has not detected the phytoplasma in affected plants.

In summary, we have found P. australiense associated with diseases of flax, cabbage tree, strawberry, and karamu. In Australia the same phytoplasma causes disease of grape and papaya, as well as strawberry. The phytoplasma is associated with rapidly lethal diseases (e.g., sudden decline in cabbage tree) and slow declines (e.g., grape-vine yellows). It has been associated with major epidemics (e.g., in flax and cabbage trees) and localised minor diseases (e.g., in strawberry). Present knowledge indicates that this phytoplasma is restricted to Australia and New Zealand.

So what can be done about it? Unfortunately, if your cabbage tree shows symptoms there is nothing we can recommend. Because accurate disease diagnosis, especially at the early stage, is difficult, we suggest leaving the tree until it is obviously affected, as shown by it losing most of its leaves, before removing it. Injecting with antibiotics is expensive and not effective unless done at the very early stage of infection. Strawberry growers manage their disease by removing affected plants to allow the neighbouring plants to expand into the gap and produce more runners. As far as managing the disease in karamu, the loss of individual plants of karamu is not an obvious problem, and indeed may enhance succession to forest. However, the possibility that karamu plants may act as sources of infection for other species is of concern. While more work is needed to confirm this possibility, we propose a precautionary approach. Specifically, we suggest that establishing virtual monocultures of karamu is undesirable, and recommend that restoration plantings include rather more diversity than is sometimes the case.

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