The threat of *Phytophthora ramorum* to woody plant collections…

IAN WRIGHT

SINCE 2003 A NEW THREAT has appeared in UK gardens, a problem that has yet to be fully understood and one which is potentially very damaging to our woody plant collections – *Phytophthora ramorum* (PR), known in the USA as 'Sudden Oak Death'.

PR is a fungus-like pathogen, belonging to a group of organisms known as the Oomycetes. Until recently they were thought to be fungi as they produce spores, have hyphae and are unable to manufacture their own food but acquire nutrition by degrading and absorbing living plant tissue. DNA analysis in the 1990s indicated that these organisms were more closely related to the algae groups (diatoms and brown algae in particular). They have since been placed in a separate taxonomic Kingdom, Chromista, as opposed to the Kingdom Fungi. Therefore *Phytophthora ramorum* is 'fungus-like'.

Infection with PR will often, but not always induce a host plant to display disease symptoms, but will not necessarily kill it. In biological terms, a 'host' is a living entity (plant or part of a plant) that the pathogen obtains its nutrients from through infection, colonisation and breakdown of the tissues. Symptoms such as death of growing shoots, loss of foliar and bleeding lesions on the bark are characteristic.

PR has a complex lifecycle completed on colonised host plants. It can rapidly reproduce asexually under favourable weather conditions (cool to mild temperature and very high humidity/rain). This type of reproduction only occurs on foliage, fruit and tender shoots of susceptible host plants. When environmental conditions are right many microscopic sporangia, which are lemon-shaped structures, develop on the surface of infected leaves. The contents of each sporangium divide up to form about 20 motile zoospores that are released from the sporangium in wet conditions. Zoospores swim in a thin film of water to healthy host tissue and infect it, thus repeating the cycle. Zoospores are therefore considered the primary form of inoculum (infectious propagules) of PR. Under favourable conditions inoculum can be produced abundantly and rapidly (within hours). This type of inoculum is not formed on the bleeding lesions on tree stems.

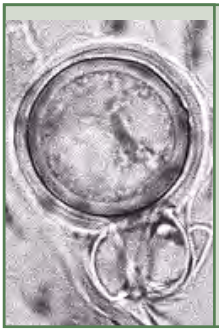
PR was first identified along the west coast of the USA in the 1990s where a close relation of the strain of the pathogen affecting the UK is devastating the American tan oak (*Lithocarpus densiflorus*) population along with other native American oak species (*Quercus agrifolia* (Coast live oak) and *Q. kelloggii* for example). The UK and US types of the pathogen are known to be different from each other. The UK population of PR is slightly more aggressive than its American counterpart, grows faster, is more stable and is comprised largely of the A1 Mating Type. The American population is less aggressive than the European population, has slower growth and two main genetic lineages, and is comprised uniquely of the A2 Mating Type. There is great concern that if the American and

PHYTOPHTHORA RAMORUM (European lineage, Mating Type A1) mature sporangium with emerging zoospores – the main form of inoculum that drives epidemics

Average diameter of zoospores 8–10µm, average length of sporangia 40–70µm

INSET Sporangium of *Phytophthora kernoviae* for comparison

FOREST RESEARCH



OOSPORE OF *P. RAMORUM* produced artificially as the result of sexual reproduction; not known to occur in nature as *P. ramorum* requires compatible mating strains (A1 and A2) to be in close contact. Average diameter 27–32µm

FOREST RESEARCH

European populations come into contact with one another they will able to sexually reproduce, creating very long-lived spores called oospores and that the progeny may be even more aggressive than the parent types.

When the disease was first noted in the UK it was identified mainly on viburnums and rhododendrons in nurseries but was later detected on these and other hosts in outdoor situations. The initial concern was – and still is – the potential risk of infection in our own woodlands and native tree species. Since the initial discovery of *Phytophthora ramorum* our understanding of the pathogen has grown and with it our apprehension.

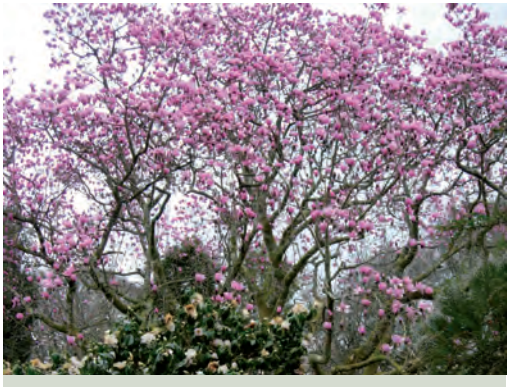
The list of susceptible plants continues to grow and now includes a wide range of woody genera other than *Viburnum* and *Rhododendron*, such as *Magnolia*, *Pieris*, *Drimys* and *Kalmia*; and many tree species, but particularly European beech, are susceptible to this pathogen. Regularly updated lists of susceptible plants in the UK are available on the UK's Department for Environment, Food and Rural Affairs (DEFRA), the EU funded Framework 6 project (RAPRA) and Forest Research and Central Science Laboratory websites.

Outbreaks of PR have been fairly widespread within nurseries in the UK, perhaps an indication of how quickly any disease can move when a pathway is available. Many nursery outbreaks, however, have been contained and eradicated successfully, due to the nature of these sites and the vigilance of the UK Plant Health Inspection Service, but it is a more difficult and costly process to control once outside the nursery walls. In the 'outdoor' situation the greatest concentration of outbreaks is in the southwest, including Cornwall and southern Wales, but the epidemic has spread across most parts of England and Wales and has now also been found in Scotland and Northern Ireland.

. . . from a Head Gardener's perspective

Sir Edward Bolitho gave Trengwainton, in southwest Cornwall, to the National Trust in 1961 after he received the Victoria Medal of Honour from the RHS for 'Services to Horticulture'. The skilled work completed earlier in the 1930s by Alfred Creek, the then Head Gardener, helped the garden win national accolades for the new hybrids and material propagated from the 1927–8 Kingdon-Ward expedition to Assam and the Mishmi hills in upper Burma.

During the last 6 years, as the current Head Gardener at Trengwainton, I have had many concerns about factors affecting our ageing plant collection. Honey Fungus (*Armillaria* spp.) in particular causes endless heartache as a result of the annual losses it causes. *Phytophthora ramorum* and the more recently discovered *Phytophthora*



MAGNOLIA CAMPBELLII, planted in 1926 at Trengwainton and as yet uninfected IAN WRIGHT

PHYTOPHTHORA RAMORUM on *Rhododendron* 'Loderi King George' IAN WRIGHT

kernoviae have now given us additional cause for concern. (Found while surveying for *P. ramorum*, *P. kernoviae* is, at the moment, predominantly Cornwall-based, as its name suggests [Kernow being the Cornish word for Cornwall]. However, scientists regard it as a more aggressive strain.)

Although our collection of plants has declined in content over the last few decades, it is still acknowledged as being of significant merit both within the National Trust and beyond.

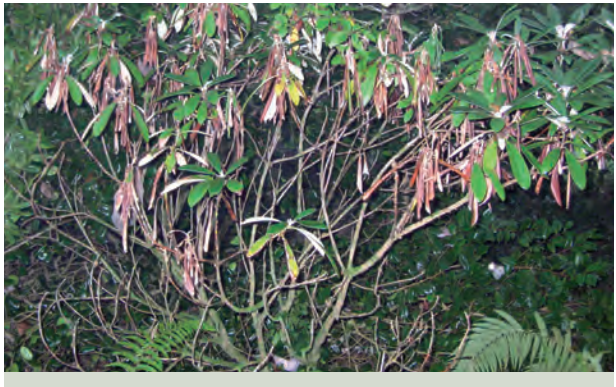
Against that background, we made a decision not to sit back and wait any longer for further damage from these or any other diseases, but to try to react proactively, identifying ways of safeguarding our collections, as well as working with others to understand the disease and manage our gardens to lessen any risk. As a result, we have looked at ways we can help affected gardens and share any good practice we learn both internally and externally.

RHODODENDRONS

Outbreaks of PR & PK have mainly been identified on the medium to smaller leaved types such as *R.* 'Fragrantissimum', *R.* 'Golden Oriole', *R.* 'Temple Belle' and the various *R. ponticum* hybrids. However there are cases on the 'Loderi' types and the risk that some of the larger leaved species such as *R. sinogrande* could also be susceptible. Susceptibility testing is now being carried out on a wide range of *Rhododendron* species. Obviously the threat to our own Trengwainton hybrids is causing us the greatest concern.

Various symptoms have been observed such as dying leaf midribs, leaves and shoots with drought-like characteristics, wet-looking fungal blotches on leaves, dead flower buds and canker lesions on stems. With smaller plants we have found that the disease will usually overwhelm the plant, defoliating then eventually killing it. Larger plants seem to struggle on, with the risk that they will succumb to a secondary disease in their weakened condition. We have found some types will temporarily shows signs of recovery, only to present with new symptoms up to 8 months later, perhaps due to weather conditions – a point I will return to later.

One of the major hosts of the disease was found to be *Rhododendron ponticum*, its huge biomass providing a host for large inoculum levels. As with other gardens in Cornwall, we are removing *R. ponticum* where possible within the garden as well as from the immediate



PHYTOPHTHORA KERNOVIAE on *Rhododendron* 'Ding Dong' – a Trengwainton hybrid

IAN WRIGHT

MAGNOLIA DELAVAYI infected with *Phytophthora kernoviae*

IAN WRIGHT

surrounding area, in order to lessen the risk to our more valuable specimens. This brings additional problems however, as a lot of the older *ponticums* were planted for shelter and indeed, like other collections, ours includes a lot of *ponticum* hybrids.

MAGNOLIAS

A wide range of our *Magnolia* species have proved positive with both of the diseases, for example *Magnolia delavayi*, *M. salicifolia* and *M.* x *soulangeana* to name but a few.

A mature *Magnolia delavayi* with *Phytophthora kernoviae*, at over 10 metres in height with a large canopy, growing in a free standing and open situation, displayed similar characteristics to some of the infected rhododendrons when it began dropping green leaves with dying lower midribs. (A sign that always raises suspicion that there may be a problem is a carpet of green leaves under a 'susceptible' evergreen plant.) As spring turned to summer, the leaf drop stopped, but resumed in the autumn. This tree has now lost around 20% of its canopy and is obviously now in a much weakened state and potentially more at risk from secondary diseases. Moreover, rain dripping from it, carrying inoculum picked up from running over infected leaf surfaces, has also killed a young *Rhododendron* 'Morvah' (a Trengwainton hybrid) growing beneath its canopy.

The trunk of the *M. delavayi* has also developed a bleeding lesion on its bark – a dark residue seeping from spots on the stems – which is an indication of infection within the growing cambium and inner bark. In general, symptoms that can be seen on the outside of a tree are indicative of only 5% of the infection beneath the bark's surface.

Similar foliar symptoms have been observed on mature plants of *M. salicifolia*. As with rhododendrons, the disease seems to come and go, depending on weather conditions.

CAMELLIAS

Camellias seem to be slightly more resistant to the disease when mature, but not when in a nursery situation. Perhaps some resistance is gained from the thicker, glossy leaves, but one should not be complacent as, if a high level of inoculum is nearby, positive cases will almost certainly be found on mature plants, and defoliation is sure to follow.

ONGOING RESEARCH

The Central Science Laboratory (CSL, York) and Forest Research (Alice Holt Lodge, Surrey) are currently undertaking various research projects to understand the disease, and a gradual picture of the cycle of the pathogen is emerging.

PHYTOPHTHORA RAMORUM causing defoliation on *Camellia* x *williamsii* 'Donation'

IAN WRIGHT

RHODODENDRON 'Morvah' at Trengwainton

IAN WRIGHT

ON-SITE IDENTIFICATION AND ANALYSIS

At present a Lateral Flow Device (LFD) is used to confirm the presence of any of the *Phytophthora* species on plant material. If the test proves positive, a sample is sent to the laboratory to test if it is in fact either *P. ramorum* or *P. kernoviae*. This process can take 10 days.

New methods are being trialled to reduce the test time and increase the identification success rate. One new system is called a Polymerase Chain Reaction (PCR) or 'Smart Cycler' which uses a magnetic process to capture clean DNA. This is then tested against known results and controls to ascertain whether the disease is either *P. ramorum* or *P. kernoviae*. This process can provide an answer in around two hours and can be used on site.

Another area of research uses laser and digital imagery to create a 3D picture and monitors how the disease spreads once a plant has become infected.

Sensors at affected sites are also monitoring temperature and check air samples for the presence of airborne *P. ramorum* or *P. kernoviae* spores.

Known pathogen-free leaves of 'susceptible' species of *Magnolia* or *Rhododendron* are used as 'bait' and placed in watercourses to establish if the water is positive for PR and/or PK.

THE INFLUENCE OF THE CLIMATE

The possibility of a link with climatic conditions is another area for research. The disease does seem to be more active in warm, wet conditions (not good news for areas with high rainfall) and become less visible in hot, dry periods such as the summer of 2006.

One can predict a resurgence of the disease to within a few days, usually around 10 days or so after a wet period starts in the spring or autumn. However Cornwall's winters are generally mild and damp and positive results have continued to be picked up throughout the season.

A PROACTIVE RESPONSE

If you don't know what a plant is, then how can you quantify its historical and genetic significance?

SURVEYING

At Trengwainton, we found we needed a more comprehensive set of records than we had assumed were in place, with large areas of our collection being left out of the Woody Plant Catalogue system we had been using. The Catalogue was very out of date, the situation being accentuated by the quick turnover of plants in this area of the country. We needed a better picture of what we were looking after and growing in our garden.

RHODODENDRON 'Johnnie Johnston' at Trengwainton

PAM HAYWARD

Using the new NT plant database, we have completed around 75% of a full survey and input this to the system. This essential knowledge has enabled us to form a Priority Propagation List of the most historically valuable specimens on the property. We can now instigate a programme to safeguard these plants before we sustain further losses.

A future aim would be to use a GPS system to digitally map the collection, in conjunction with a programme of fixed point photography and we will also be revisiting our conservation plan for the garden to include ideas for temporary planting to fill areas where losses have occurred.

HYGIENE

The threat from the disease has also made us review our general garden hygiene. Working from plant to plant within a garden situation can potentially spread this or indeed any disease.

Various disinfectants are suggested for use; obviously the stronger the product, the more risk to the operator and the more restrictions from government regulations, even though in most cases these products will only form a barrier, not kill the disease. Washing tools and boots etc. on a regular basis is recommended and this good practice should become force of habit.

Sweeping up and burning fallen diseased leaves is also essential to help keep inoculum levels down.

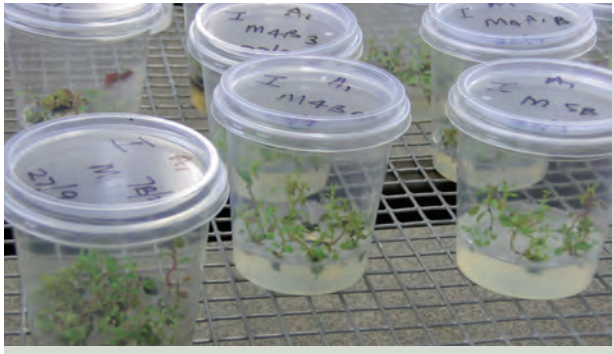
Being a garden open to the public, we are satisfied that there is little risk of spread from visitors to our garden. The normal requests we make of any visitor, for example: keeping to the paths, dogs on leads and non-removal of plant material should significantly lessen any risk. We have also stepped up our path cleaning maintenance operation.

Initial trials are underway with a chemical called phosphonate which may, in the future, offer some protection to historic plant material. However, this is very much in the preliminary stages of trial and research.

PROPAGATION

When the disease is confirmed within the boundaries of a garden it becomes more difficult to employ some standard plant propagation options due to the risk of spreading the disease by the movement of untested green material, for example.

We also discovered we had been watering young replacement plants in our garden nursery with water which has since been tested *P. ramorum* positive.



RHODODENDRON 'Morvah' plantlets in the Duchy College Micropropropagation Unit IAN WRIGHT

The traditional propagation procedure within the National Trust is via the central Plant Conservation Programme nursery located at Knightshayes in Devon, but this route is difficult when infection is confirmed. We have consequently been working with Duchy College at Rosewarne in Cornwall to micropropagate our more valuable specimens. (Micropropagation is the production of a potentially infinite number of plants by using bud or shoot tissue in a controlled solution usually within a laboratory type situation.) So far, they have had considerable success in encouraging buds to respond to the process within laboratory conditions and it is hoped that, once growing, the infection-free material could itself be propagated and then passed on to Knightshayes. In an ideal situation we would hope that enough plants are produced to distribute to other clean gardens with similar growing conditions to our own. The micropropagation unit has achieved a good

success rate with our Trengwainton rhododendron hybrids such as 'Morvah', 'Creeks Cross' and 'Johnnie Johnston'.

Buds from both *Magnolia delavayi* and *M. campbellii* ssp. *mollicomata* have also shown initial signs of response. We remain hopeful that our number one priority plant, *Rhododendron macabeanum*, will in time respond to this process. *R. macabeanum* KW7724 flowered for the first time at Trengwainton from material brought back by Frank Kingdon-Ward from his plant hunting expeditions to Assam and the Mishmi Hills in upper Burma in 1927–8. It was awarded a First Class Certificate from the RHS in 1938.

THE MICROPROP PROCESS

Plants are cultured in an agar jelly containing specific nutrients and hormones, specially formulated to be appropriate to the material type and the desired result i.e. whether it is intended to increase numbers through shoot generation/multiplication or rooting. Samples are first disinfected to remove any competing or parasitic microbes, like bacteria and fungi which are detrimental to the plant. This is achieved by using a surface sterilant – usually a hypochlorous solution or alcohol. The action of these is enhanced by the addition of wetting agents which improve surface contact with the sterilant. The process often involves multiple stages with various concentrations and treatments.

Once samples are clean and shoot multiplication has been stimulated, the number of plants can increase exponentially. Micropropagation is typically used for commercial propagation on a large scale, but the same methods can also be used to multiply rare and threatened plants.

The technique of micropropagation has many merits when dealing with plant pathogens such as *P. ramorum*. Firstly, one can produce a large number of plants from a small quantity of parent material and, once established, continue to produce them as required. Secondly, it provides a high degree of confidence in pathogen elimination in the optimum growth conditions which are provided. Any infection rapidly multiplies to the point where the colony is visible and if no contamination is observed after an extended period the assumption can be made that decontamination was successful.

Some problems with the process still remain such as:

DECONTAMINATION

Plant material, which has matured *in vivo*, is typically unsuitable for micropropagation since total decontamination is virtually impossible. It doesn't take long in the outside world for a sample to become riddled with fungal spores, algae and bacteria. This is especially relevant in the protected microclimates that occur in the old walled gardens at Trengwainton where many of these varieties are planted. Unfortunately, without clean stock plants, this is the only propagation material available for much of the year. To sufficiently clean even very young material may require a concentration of sterilant so high it may prove phytotoxic.

CULTIVAR VARIATION

The wide variation between cultivars means that a treatment that has produced significant results with one sample may have absolutely no effect on another. This makes it difficult to produce a definitive protocol for propagation because of varying responses. Paradoxically however, it isn't a viable proposition to produce a different protocol for each cultivar because then the methods are too complex to replicate.

OXIDISATION

A problem found with camellias is that damaged tissue oxidises (seals over) very readily. This then inhibits water and nutrient uptake, and some of the exudates (natural sealants) can even cause the tissue to degrade.

SLOW GROWTH RATE

Ancient plants often demonstrate a gradual decrease in vigour as they age, so the rate of growth in material taken from a plant of 10–20 years old is considerable when compared to samples taken from plants aged over 150 years.

A WAKE-UP CALL

P. ramorum is proving to be a significant 'wake-up call' for our gardens and although one could argue that if it does prove to be 'just another

problem that we have to live with', there might just as easily be another disease, pest or extreme weather condition awaiting its turn in the future.

There is a continuous danger of introducing new pathogens into our gardens when we bring in exotic plants or nursery plants produced in different countries where they may have been exposed to alien pests and microbes. We will need to reassess how we are to maintain the beauty and variety in our gardens without threatening our current collections.

By evaluating the management of our garden and plant collection, the changing climate and the seemingly endless stream of new pests and diseases, and also taking into consideration the resource level and our evolving roles, it is apparent that ideally we need to spend more time giving the ageing plants within the collection the annual care they need, i.e. mulching, pruning or feeding, especially since the health of some of the collection has been compromised by the loss of canopy cover after the two storms of 1987 and 1990. It has made us look more closely into our aftercare of young plants and made sure we continue to rejuvenate and propagate our older, historically-valuable specimens. I firmly believe in the old analogy that healthy plants would stand a better chance if threatened by *Phytophthora*, or any other disease. We will certainly plant less and look after more in the future.

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Further, more detailed information on PR and PK can be found on the following websites:

www.defra.gov.uk/planth/pramorum.htm
www.forestry.gov.uk
www.rapra.csl.gov.uk

email: planthealth.info@defra.gsi.gov.uk

With thanks to Sandra Denman from Forest Research and Peter Hodgson from Duchy College for their help with the sections on PR and PK, and micropropagation respectively.

We would be grateful if anybody has reference to, or has living material of, the following rhododendrons that we believe were originally bred or raised at Trengwainton.

'Beta' A selection from *R. lanigerum* KW8251; Bolitho 1963; rose-opal.

'Bulldog' (*elliottii* x 'Earl of Athlone'); Bolitho 1937; deep red.

'Cherubim' (*Azma* x *elliottii*); Bolitho 1943.

'Cornish Cream' (*campylocarpum* x *Fortorb*); Bolitho 1937.

'Cornish Glow' (Lanarth hybrid x Lanarth hybrid); Bolitho 1947; crimson changing to orange yellow.

'Ding Dong' (*fortunei* ssp. *discolor* x *lacteum*); Bolitho pre 1958; white.

'Johnnie Johnston' (*johnstoneanum* (double form) x *tephropeplum*); Bolitho pre 1958 – we need to bulk up our few remaining specimens.

'Laerdal' (*johnstoneanum* x *dalhousiae*); Bolitho 1937; pure white.

'Lal Kapra' (*neriiflorum* x *sanguineum*); Bolitho 1958; red.

'Lanyon' (*haematodes* x *elliottii*); Bolitho pre 1958.

'Miss Pink' (Azma Group x *griersonianum*); Bolitho 1943.

'Morvah' (*elliottii* x *wattii*); Bolitho pre 1956; turkey red.

'Nanceglos' (*elliottii* x *fortunei*); Bolitho 1945.

'Penalverne' ('Earl of Athlone' x *griersonianum*); Bolitho pre 1958.

'Penhale' (*fortunei* x *facetum*); Bolitho 1945.

'Rubeotinctum' A selection from *R. johnstoneanum* KW7732; Bolitho 1941; white with a deep pink stripe on each lobe and a pink or yellow blotch.

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