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New Zealand Bacteria, Viruses and Zoospores.

Bacteria

ESR scientist Dr Brent Gilpin explains that bacteria are everywhere, and there are billions of them around us all the time. Only a small number are harmful to health, and it is identifying those in a background of harmless bacteria that is a challenge to scientists.

Transcript
DR BRENT GILPIN
Bacteria exist everywhere on earth. I mean in our… each of our intestinal systems, we've got more bacteria than there are people living on earth. So amongst those, there are thousands and thousands of different types, and so we are literally swimming in bacteria all the time. It's a very small proportion of these that actually cause disease – most of them are beneficial to us. Indeed, life on earth as we know it would stop without all these organisms. So when you are trying to identify specific organisms, you are really looking at one or two amongst a whole background of other organisms, and they are very good at adapting, so they will change their system so they are able to live in new hosts and adapt to new conditions. So there is a constantly changing bacterial population going on, so when we go to look at our faecal source identification tools, we've got to really sift through a wide range of bacteria, some of which will only be transiently present, some which are present in very low levels, and others which may be present in a whole range of organisms, some which may not be present in faecal material, some which may grow in the environment and be found in other places. So for faecal source discrimination, you've really got to find just those bacteria or other organisms which are only present in faeces and, ideally, only present in a particular animal source.
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ESR scientist Dr Brent Gilpin explains that a bacteriophage, or phage, is a virus that infects bacteria. He describes how a phage can be easily detected in the lab by plating bacteria on an agar plate. When clear spots appear on a 'lawn' of bacteria, it shows that the phage has infected and killed areas of bacteria.

Transcript
DR BRENT GILPIN
A bacteriophage, or a phage, is a virus that infects bacteria, so these don't infect human cells –they are very specialised and only infect bacteria. So every bacteria is being attacked by various viruses which are using those as the host that they replicate in. The bacteriophages are different to the eukaryotic viruses because, to detect a virus that infects your eye, ideally you need to infect it into your eye, or else a human cell tissue culture line, which isn't exactly the same as your eye. Whereas a bacteriophage infects bacteria, and bacteria we can grow in the laboratory rather easily. So the entire organism can be grown on a plate, and if you add a mix of bacteriophage with the bacteria, you will see on the plate which doesn't have bacteriophages it will be a lawn of bacteria, whereas wherever there is holes in that plate of bacteria, it indicates that a bacteriophage has infected those and has killed all the bacteria in the vicinity, which is effectively what bacteriophages do. They get into the cell, reproduce themselves and then bust that cell open, killing the bacteria, and then go and find some more hosts to infect.

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Zoospores

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Susceptibility of New Zealand flora to Phytophthora ramorum and pathogen sporulation potential: an approach based on the precautionary principle

A study like the one described here is only a first step towards the identification of potential host and geographic ranges favourable to this pathogen in New Zealand, it does have the obvious advantage of avoiding any of the risks associated with importing the pathogen to New Zealand for experimentation. The number of New Zealand species and the number of genotypes per species available at U. C. Berkeley was obviously limited. Under these constraints, we propose caution regarding species that were negative in foliar and branch inoculations.

Furthermore, those species that were positive should be accepted as putative hosts pending a comprehensive study to determine whether resistance plays a role (see Tooley et al. 2004; Dodd et al. 2005; Hayden and Garbelotto 2005; Hüberli et al. 2006a). Nonetheless, the positive results obtained here are likely to be highly correlated with the genetic makeup of the tested species, due to the fact that all plants were from the U. C. Berkeley botanical garden and, hence, differential site effects on disease development were reduced to a minimum, as expected for a common garden experiment.

One approach we adopted to bypass the issue of artificial results caused by artificial conditions in the laboratory, was that of checking which plant species were susceptible in vitro when using low, but maybe more realistic, zoospore concentrations. At low inoculum concentrations, disease dropped markedly for all species including the known susceptible Rhododendron cultivars, but not for F. excorticata. Results from the low inoculum concentration inoculation thus identified F. excorticata as extremely susceptible. At higher inoculum, F. excorticata even surpassed the susceptible Rhododendron cultivars in susceptibility. Since disease was unrelated to inoculum load for F. excorticata, this potential host is probably extremely susceptible, and we recommend it is used as an indicator for early detection of P. ramorum infection in high risk areas of New Zealand. Previous studies found that detached foliar tests are often affected by host physiological factors, including leaf age and position in the canopy (Denman et al. 2005; Hansen et al. 2005). In our foliar assay, we only used mature leaves as these are less susceptible than juvenile leaves (Denman et al. 2005; Hansen et al. 2005). Additionally, leaves were always chosen from the sunny side of a plant, where the potential for epidermis formation was highest. Incubation in darkness in our foliar assay was unnatural and may have contributed to lower resistance because leaves could not photosynthesise as stomatal opening was affected (see Tooley et al. 2004). Detached branch inoculations are widely used to assess susceptibility (Dodd et al. 2005). Despite the artificial nature of our inoculation assays, we showed susceptibility among species tested in excised branches and leaves. When available, whole plants exposed to natural inoculum in the field are the best substitute for detached leaves or branches, as they provide holistic plant responses.

All our inoculations used the A2 mating type isolate, Pr-52. We chose this isolate because it had a known pathogenicity from our previous studies (Hüberli et al. 2006a) and also for comparability to other studies that used this isolate on several hosts with differing inoculation methods (Rizzo et al. 2002; Tooley et al. 2004). Whilst quarantine regulations prohibited using the A1 isolate in our inoculations, other foliar inoculation studies using both A1 and A2 mating types found no statistical difference in aggressiveness amongstisolates (Tooley et al. 2004; Denman etal.2005). Clear differences in aggressiveness amongst the A1 and A2 mating type isolates occurred in log inoculations (Brasier 2003) but, as far as we are aware, never in foliar inoculations.

Overall, while our experiments do not definitively establish the extent of susceptibility of the New Zealand flora to P. ramorum, they do show clearly the potential for the spread of any introduction, with possibly severe economic and conservation consequences. This argues for strong precautionary measures.

What precautionary measures should be applied?
New potential hosts were identified in this study. These should be included in monitoring programs not only in New Zealand, but in all places where P. ramorum is regarded as a dangerous pathogen and regulated. Given some plants display no symptoms, the presence of P. ramorum should be screened, not only by surveying for symptoms, but also by destructive sampling of a small number of plants to be assessed using the most sensitive available DNA-based assays (for instance Hayden et al. 2006 or Hughes et al. 2006). In addition, soil baiting of a subset of remaining plants could be used to identify the presence of the pathogen. Intensive screening of potential Australian hosts to P. ramorum is underway to identify hosts with possible high sporulation potential and to develop incursion risk models (Hüberli et al. in press).

Our current study lists potential hosts, both foliar and stem, which may place New Zealand'snative forest and the commercial plantations at risk of a P. ramorum incursion. While few indigenous plants were tested for susceptibility to P. ramorum, 12% (2/17) of them showed

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substantial susceptibility. Areas where F. excorticata and N. fusca are sympatric were further identified as zones extremely favourable to invasion by this pathogen. Were this pathogen to be introduced in New Zealand, it is these areas that could witness the development of a forest epidemic comparable to sudden oak death inCalifornia. More species should be screened to accurately assess the risk posed to New Zealand and the movement of these foreign plant species within USA and Europe. Until such time, in accord with New Zealand flora Australasian Plant Pathology 623 the precautionary principle, it is prudent to implement strict quarantine measures in an attempt to prevent an accidental introduction of P. ramorum.