Mycologist, Volume **18**, Part 4 November 2004. ©The British Mycological Society Printed in the United Kingdom.
DOI: 10.1017/S0269915XO4004136

Phytophthora ramorum: one pathogen and many diseases, an emerging threat to forest ecosystems and ornamental plant life

ALEX A. APPIAH[§], PHIL JENNINGS & JUDITH A. TURNER

Environmental Entomology and Plant Pathology, Central Science Laboratory, Sand Hutton, York, YO41 1LZ UK.
[§]Corresponding author: Dr. Alex A. Appiah, Tel: 01904 462094 Fax: 01904 462111 Email: a.appiah@csl.gov.uk

Phytophthora ramorum is a recently described species responsible for sudden oak death syndrome and also causes symptoms such as twig wilt and dieback, stem lesions, necrosis of leaf midrib from the petiole and leaf tip necrosis on a range of ornamental plant species. In the USA, a reported epidemic of *P. ramorum* infections on trees belonging to several families including Fagaceae, Lauraceae and Ericaceae seems to be increasing and there are fears of similar epidemics occurring in woodlands in the UK and mainland Europe. This paper reviews the current state of knowledge and the research efforts being made to understand the biology, manage the disease and prevent widespread outbreaks of *P. ramorum* infections across Europe and the USA.

Keywords: sudden oak death, quarantine organism, oomycete

Introduction

Phytophthora ramorum (Werres, De Cock & Man in't Veld), the causal pathogen of sudden oak death, was first described as a new *Phytophthora* species on *Rhododendron* and *Viburnum* in Germany and the Netherlands (Werres *et al*., 2001). On these plants, *P. ramorum* caused twig blight, which killed both nursery plants and mature bushes in gardens. *P. ramorum* is characterised by the production of abundant large chlamydospores, and elongated, deciduous, semi-papillate sporangia with short pedicels. Morphologically, *P. ramorum* sporangia resemble those of *P. palmivora*, but the sequences from the internal transcribed spacer (ITS) regions of the rDNA of *P. ramorum* isolates were closest to that of *P. lateralis* (Werres *et al*., 2001). The diseases caused by *P. ramorum* were drawn to the world's attention when large numbers of *Quercus* species including coast live oak, black oak, shreve oak, and canyon live oak, and *Lithocarpus densiflorus* (tan oak) were killed along a 300 km stretch of the California coast in the USA (Knight, 2002; Rizzo *et al*., 2002).

Geographical distribution and host range

Presently, *P. ramorum* infections in field and nursery plants have been reported in several countries including the USA (Rizzo *et al*., 2002) and Canada (Anon, 2003). In the USA, *P. ramorum* infection is currently thought to range from Big Sur in Monterey County to southern Oregon, with the furthest inland site in Western Solano County (Goheen *et al*., 2002; Kelly, 2002), and the epidemic now covers over 600 km^2 of woodland (Davidson *et al*., 2002). Over 30 natural host plants, predominantly tree species from the Fagaceae, Lauraceae and Ericaceae, as well as hybrids of *Viburnum* species, are known to be susceptible to *P. ramorum* in the USA. In Europe, countries such as Germany, the Netherlands, Belgium, Denmark, France, Slovenia, Spain, and Sweden have reported cases of *P. ramorum* infection. *P. ramorum* infections have been reported in England, Wales, the Channel Islands and the Republic of Ireland. (cited from Sansford *et al*., 2004). Presently, 31 susceptible host trees and ornamental species predominantly from the Ericaceae, Rhamnaceae and Fagaceae, and over 80 hybrids of *Rhododendron*, have been reported in UK and the Republic of Ireland, and 9 host species in other European countries (Sansford *et al*., 2004).

Significantly, the outbreaks of *P. ramorum* in Europe were predominantly on ornamental plant species such as *Rhododendron*, *Viburnum*, *Pieris*, *Camellia* and *Kalmia* in nurseries and a few public gardens. However, there have been recent confirmations of infection on southern red oak (*Quercus falcata*), beech (*Fagus sylvestris*), sweet chestnut (*Castanea sativa*), horse chestnut (*Aesculus hippocastrum*) and holm oak (*Quercus ilex*) in UK and *Quercus rubra* in the Netherlands (Sansford *et al*., 2004). In the UK, over 300 outbreak sites have been identified across England and Wales since February 2002, of which approximately 40 are in natural/semi natural situations, often in large managed gardens. The outbreak sites are scattered across the UK and there is no apparent pattern to the occurrence of the disease however, the south-east and south-west of England have sites that appear to be more heavily infected. In experimental tests, the most susceptible trees found in the UK included the mediterranean holm oak (*Q. ilex*), northern red oak (*Q. cerris*), european beech (*Fagus salvatica*), sweet chesnut (*Castanea sativa*), douglas fir (*Pseudotsuga menziesii*) and sitka spruce (*Picea sitchensis*) (cited from Sansford *et al*., 2004). The list of natural host plants and species potentially susceptible to *P. ramorum* continues to increase as new hosts are being reported from field surveys and inoculation experiments in the infected countries. However, there has not been any reported incidence of *P. ramorum* infections from other parts of the world.

Symptoms and disease transmission

On larger trees such as oak (Fig 1a), cankers caused by *P. ramorum* produce brown or black discolouration of the bark (Fig 1b), ooze dark red sap (Fig 1c), and can be found on the trunk up to 20 m above the ground (Rizzo *et al*., 2002). Cankers, can be over 2 m in length, delimited by thin black zone lines in the inner bark and do not generally enlarge below the soil line into the roots (Fig 1b). Leaves on infected trees turn from green to brown over a period of several weeks. Several symptoms of *P. ramorum* infections have been recorded on different non-tree woody plant species. These include twig wilt and branch dieback (Fig 1d), stem lesions (Fig 1e, h), necrosis of the midrib from the petiole (Fig 1f), leaf tip necrosis (Fig 1g), and aerial dieback (Fig 1i).

Natural infection and spread of *P. ramorum*, as in all oomycete species, depends on rainfall and high humidity over a period of days. Swiecki and Bernhardt (2002) reported a significant positive association between *P. ramorum* canker infection and high stem water potential in coast live oaks, indicating that rapidly growing trees in wetter soils were more susceptible to infection than those located in drier sites. Also, the foliar infections of *P. ramorum* on many plant species, particularly tan oak in the USA (Rizzo *et al*., 2002) and rhododendron in the UK, have been implicated as important sources of inoculum for tree infection. When washed into soils, sporangia and chlamydospores produced in leaf and twig lesions of susceptible understorey hosts, contaminate leaf litter and soil and can subsequently infect host seedlings and mature plants. Davidson *et al*. (2002) suggested that wind-blown rain or rain splash dispersed inoculum is responsible for long distance movement of spores for new infections. Also, *P. ramorum* has been recovered from rain, soil, litter and streams from woodland with infected oak and Californian bay laurel trees (Davidson *et al*., 2002). Spread of the disease through movement of contaminated materials such as soil, logs, wood and ornamental plants through human and animal activities has been reported (Tjosvold *et al*., 2002). The potential for transmission by vectors, such as vertebrates, has yet to be demonstrated but must be considered a risk, particularly as it has been reported that dispersal is potentially occurring in the USA through spread of infected soil on hikers' boots.

Environmental and economic impact

There is a potential for tremendous socio-economic and environmental impacts in countries with reported incidence of *P. ramorum* infections in the 'wild' such as the USA and UK. These include loss of wildlife habitats, food sources and recreational areas in woodlands with high *P. ramorum* tree infections. There are also high risks of forest fire (as a result of dead infected trees), accelerated water runoff, soil erosion and sedimentation, endangering of certain plant species, and high monetary losses to the multi-billion dollar timber and horticultural industries. High costs are associated with the control strategy of removing dead trees, with the level depending on the location of the infected plants. In Europe, where *P. ramorum* infections have been found mainly on commercial and containerised plants, and the pathogen is subject to phytosanitary controls, the economic impact may be largely weighed against the cost associated with official controls such as surveillance, eradication, and containment (Sansford *et al*., 2004), as well as losses incurred by horticultural establishments whose plants become infected. There is also the potential danger of development of aggressive hybrids through sexual recombination between the European isolates (A1

Fig 1 Symptoms of *Phytophthora ramorum* diseases in plant species. **a)** Sudden Oak Death canker infection on *Quercus falcata* (Southern red oak). Arrows show extensive cankers that have girdled and killed the tree, **b)** Enlarged picture of insert from **Fig 1a** showing scrapped bark surface of *Q. falcata* revealing canker [black or dark brown discolouration of the bark] lesions, \ Seeping dark red sap from cankers (arrowed), **d)** Twig wilt and branch dieback on *Rhododendron*, e and **h)** Dark brown stem lesions (arrowed) on *Rhododendron*, **f)** Leaf necrosis (arrowed) of the midrib beginning from the petiole, **g)** Leaf tip necrosis on *Kalmia*, **i)** Aerial dieback of *Rhododendron* bush, and **j)** and **k)** Re-infection of *Rhododendron* regrowths on coppiced previously infected bush. [Photographs provided by the courtesy of Mr. Stephen Eales, PHSI (Defra)].

mating type) and the American isolates (A2 mating type), if both are introduced into the same region.

Current Management Strategies

The management strategies currently practiced, particularly in Europe, rely heavily on eradication and phytosanitary measures. These measures which are supported by national and international legislation, include a ban on importation of susceptible plants and wood from infected areas in the USA, and plant passport controls for the movement of certain susceptible plants within the European Community. These movement controls are supported by a comprehensive official inspection regime including both commercially traded plants and more general surveys of plants in the wider environment. Official measures are required to eradicate all findings of *P. ramorum*. These measures include destruction of infected plants, all susceptible plants within 2m of an infected plant and any associated crop debris, and the disinfection of standing surfaces. Also, all plants within 10m are held for 3 months. Although, preliminary studies on chemical (metalaxyl, copper sulphate and phosphoric acid) control of *P. ramorum* were positive in the USA, they seem to be only useful in urban situations and for the protection of specimen trees (Garbelotto *et al.*, 2002a). Cultural and chemical control, along with disinfection of irrigation systems in nurseries seem to be feasible options for reducing infection of non-tree susceptible hosts.

Current Research Activities

Various research projects have been initiated to understand the biology and population genetics of *P. ramorum* including development of rapid and molecular diagnosis of *P. ramorum* infection, determination of the complete host range of the pathogen, identification of the factors influencing survival of the pathogen and investigation of disease transmission dynamics. Also, there is ongoing work monitoring *P. ramorum* occurrence in forest ecosystems, and in developing effective management strategies for disease outbreaks. Significant progress has been made in these studies. These include direct PCR detection of *P. ramorum* on symptomatic plant materials based on ITS sequence analysis (Garbelotto *et al.*, 2002b) and detection from pure cultures based on mitochondrial sequences of cytochrome c oxidase subunit (Cox) I and II genes (Martin *et al.*, 2004). Also, population genetic analyses on US and European isolates have shown that they belong to two different sub-populations (Garbelotto *et al.*, 2002b) and a rapid distinguishing test based on single nucleotide polymorphism protocol has been developed (Kroon *et al.*, 2004), which is important for quarantine control. Mating type studies on isolates from the two different subpopulations have been performed to establish if they can successfully cross. Werres & Zielke (2003) reported that isolates from the two sub populations could not cross *in vitro* on carrot piece agar and *Rhododendron* agar, but successfully produced oospores in all *in vivo* pairings after 5-8 days incubation of infected plant tissue on agar plates. The potential host range of *P. ramorum* has been determined through stem inoculation of mature forest and plantation trees (Brasier *et al.*, 2002; Hansen & Sutton, 2002) and by inoculation of detached wounded leaves of ornamentals, hedgerow and woodland under storey plants (Inman *et al.*, 2002; Parke *et al.*, 2002) in order to establish the risk of spread in a specific ecological area. In California, digital high-resolution imagery has been used to identify dead and dying trees, and follow the progressive changes in woodlands over time (Kelly, 2002).

Work carried out in the UK has examined a number of fundamental aspects of the epidemiology of *P. ramorum* including factors affecting sporulation, dispersal, germination, infection and survival. Development of eradication and containment strategies is ongoing and research is continuing to monitor the effectiveness of current strategies and influence further refinements. Aspects of containment and control of *P. ramorum* in nurseries are being investigated through funding from the Horticultural Development Council to examine fungicide efficacy. In addition to risk management strategies for ornamentals, contingency plans for potential outbreak scenarios outside nurseries are being continually developed and evaluated. These include outbreaks on both trees and non-tree hosts, e.g. wild rhododendrons or heathland plants. The work will contribute to a better understanding of risk, the development of management strategies and contingency plans, and will provide a sound scientific basis for the development of UK and EU Plant Health Policy. Ongoing monitoring of natural outbreaks in the UK has shown that *P. ramorum* can survive the UK winter in soil and leaf litter. Re-infection of rhododendron regrowths (Fig 1j, k) from coppiced stumps of previously infected bushes, has been repeatedly observed in the field. Also, leaves lying on top of surfaces of stumps of coppiced rhododendron bushes have been found to be infected with *P. ramorum*. Further work is in progress to isolate *P. ramorum* from inner tissues of woody stumps in order to determine if infective propagules survive in woody tissue. If confirmed, it may mean such propagules could contribute as source of inoculum for infection of new growth, and will have implications for the currently practised strategies aimed at eradication and containment of the disease. Strategies for removal or reduction of sources of inoculum for re-infection of new growth in managed gardens are being investigated. Spore trapping in rain and aerial traps has not demonstrated aerial movement of spores. Monitoring of watercourses has shown extensive contamination of some of the outbreak sites, although in some cases there have been no new plant infections for over 12 months.

Conclusions

There are still many unanswered questions about the origin, long distance and cross country spread of *P. ramorum*. The levels of *P. ramorum* contamination detected at some non-nursery outbreak sites in the UK raise fresh questions about the pathogen's origin and the general notion of a possibly recent introduction of the pathogen from continental Europe. The aggressive nature of *P. ramorum* and the wide and increasing host range of the pathogen makes it a potential threat to the forest ecosystem especially areas with large concentrations of susceptible hosts species such as beech and oaks. The extent of infection in *Rhododendron* species (the most susceptible ornamental plant) in managed gardens where the general public have access makes the spread of *P. ramorum* to woodlands with susceptible trees a potential danger. The potential of the American and European isolates crossing in nature is also a cause for concern as this could lead to the development of hybrids with devastating consequence. Therefore, the development of a rapid distinguishing test for both sub groups and the enforcement of rigorous quarantine measures will be crucial in preventing the meeting of both mating types in the same region. Nonetheless, more work and research funding are required to fully understand the potential impact of *P. ramorum* infections on the British ecosystem and develop strategies to avoid a potentially major epidemic occurring in forest woodlands.

Acknowledgements

We would like to acknowledge research funding provided by the Department of Environment, Food and Rural Affairs (Defra) and the Horticultural Development Council (HDC). We also thank Dr. David Slawson and Mr. Stephen Eales, Plant Health and Seeds Inspectorate (Defra) for the helpful comments and the supply of photographs respectively, and the members of the Plant Pathology research team at CSL for their support on the various projects.

References

Anon. (2003). Canadian Food Inspection Agency. Sudden Oak Death discovered at British Columbia nursery. http://www.inspection.gc.ca/english/toce.shtml.

Brasier, C. M., Rose, J., Kirk, S. A. & Webber, J. F. (2002). Pathogenicity of *Phytophthora ramorum* isolates from North America and Europe to bark of European *Fagaceae*, American *Quercus rubra* and other forest trees. *Abstract, Sudden Oak Death Science Symposium, Monterey, California, 15-18 December 2002*. http://danr.ucop.edu/ihrmp/sodsymp/paper.

Davidson, J. M., Rizzo, D. M., Garbelotto, M., Tjosvold, S. & Slaughter, G. W. (2002). *Phytophthora ramorum* and and Sudden oak death in California: II. Transmission and Survival. *Proceedings of the Fifth Symposium on Oak Woodlands: Oak Woodlands in California's Changing Landscape*. pp 741-749. R. B. Standiford, D. McCreary, K. L. Purcell (technical coordinators). 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Garbelotto, M., Rizzo, D. M. & Marais, L. (2002a). *Phytophthora ramorum* and Sudden oak death in California: IV. Preliminary studies on chemical control. *Proceedings of the Fifth Symposium on Oak Woodlands: Oak Woodlands in California's Changing Landscape*. pp 811-818. R. B. Standiford, D. McCreary, K. L. Purcell (technical coordinators). 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Garbelotto, M., Rizzo, D. M., Hayden, K., Meija-Chang, M. Davidson, J. M. & Tjosvold, S. (2002b). *Phytophthora ramorum* and Sudden oak death in California: III. Preliminary studies in pathogen genetics. *Proceedings of the Fifth Symposium on Oak Woodlands: Oak Woodlands in California's Changing Landscape*. pp 765-774. R. B. Standiford, D. McCreary, K. L. Purcell (technical coordinators). 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Goheen, E. M., Hansen E. M., Kanaskie, A., McWilliams, M. G., Oaterbauer, N. & Sutton, W. (2002). Sudden oak death caused by *Phytophthora ramorum* in Oregon. *Plant Disease* **86**: 441.

Hansen, E. M. & Sutton, W. (2002). Log inoculations to assess tree susceptibility to sudden oak death. *Phytopathology* **92**: S33.

Inman, A. J., Beales, P. A., Lane, C. R., & Brasier, C. (2002). Comparative pathogenicity of European and American isolates of *Phytophthora ramorum* to leaves of ornamental hedge row and woodland understorey in plants in the UK. Abstract, Sudden Oak Death Science Symposium, Monterey, California, 15-18 December 2002. http://danr.ucop.edu/ihrmp/sodsymp/paper.

Kelly, N. M. (2002). Monitoring sudden oak death in California using high resolution imagery. *Proceedings of the Fifth Symposium on Oak Woodlands: Oak Woodlands in California's Changing Landscape*. pp 799-810. R. B.

Standiford, D. McCreary, K. L. Purcell (technical coordinators). 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Knight J. (2002). Fears mount as oak blight infects redwood. *Nature* **415**:251.

Kroon, L. P. N. M., Vestappan, E. C. P., Kox, L. F. F., Flier, W. G. & Bonants, P. J. M. (2004). A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*. *Phytopathology* **94**: 613-620.

Martin, F. N., Tooley, P. W. & Blomquist, C. (2004). Molecular detection of *Phytophthora ramorum*, the causal agent of SuddenOak Death in California, and two additional species commonly recovered from disease plant material. *Phytopathology* **94**: 621-631.

Parke, J. L., Hansen, E. M. & Linderman, R. G. (2002). Sporulation potential of *Phytophthora ramorum* on leaf disks from selected host. Abstract, Sudden Oak Death Science Symposium, Monterey, California, 15-18 December 2002. http://danr.ucop.edu/ihrmp/sodsymp/paper.

Rizzo, D. M., Garbelotto, M., Davidson, J. M. & Slaughter, G. W. (2002). *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* **86**: 205-214.

Sansford, C., Jones, D. R. & Brasier, C. M. (2004). Revised Datasheet for *Phytophthora ramorum*. 2 January 2004. PPP 9602. Internal CSL document.

Swiecki, T. J. & Bernhardt, E. A. (2002). Evaluation of Stem Water Potential and other tree and stand variables as risk factors for *Phytophthora ramorum* canker development in coast live oak. *Proceedings of the Fifth Symposium on Oak Woodlands: Oak Woodlands in California's Changing Landscape*. pp 787-798. R. B. Standiford, D. McCreary, K. L. Purcell (technical coordinators). 2001 October 22-25; San Diego, CA. Gen. Tech. Rep. PSW-GTR-184. Albany, CA: Pacific Southwest Research Station, Forest Service, US Department of Agriculture.

Tjosvold, S. A., Chambers, D. L., Davidson, J. M. & Rizzo, D. M. (2002). Incidence of *Phytophthora ramorum* inoculum found in soil collected from a hiking trail and hikers' shoes in a California park. Sudden Oak Death Science Symposium, Monterey, Ca., Dec 15-18, 2003. http://danr.ucop.edu/ihrmp/sodsymp/poster/poster46.html.

Werres, S. & Zeilke, B. (2003). First studies on the pairings of *Phytophthora ramorum*. *Journal of Plant Diseases and Protection* **110(2)**: 129-130.

Werres, S., Marwitz, R., Man in't Veld, W. A., de Cock., A. W. A. M., Bonants, P. J. M., de Weedt, M., Themann, K., Illieva, E. & Baayen, R. P. (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Virburnum*. *Mycological Research* **105**: 1155-1165.