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REVIEW

A California-based chronological review (1995–2004) of research on *Phytophthora ramorum*, the causal agent of sudden oak death

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Summary. This review describes in chronological order the events surrounding the discovery in California of the causal agent of the forest disease known as Sudden Oak Death. Advances in the understanding of this emergent disease have occurred over a very short period of time and include elements of host-pathogen interactions, epidemiology, genetics, as well as the development of treatment options. Only three years from its discovery in California, the entire genome of *Phytophthora ramorum* was sequenced. The availability of the genome offers endless possibilities for research, and it has already been tapped to provide the strongest evidence yet in support of an exotic nature of this pathogen both in California forests and in European nurseries. Finally, this emergent disease highlights the inevitable connectivity between the ornamental plant business and the health of native forests.

Key words: plant disease, exotic pathogen, aerial phytophthora, molecular analysis, chemical control.

This review describes in chronological order the events surrounding the discovery in California of the causal agent of the forest disease known as Sudden Oak Death. We hope it will not only provide the reader with a basic synopsis of what is known about this novel pathosystem, but also relate the human and societal aspects of a unique experience for a group of scientists involved in highly visible research. Advances in the understanding of this emergent disease have occurred over a very short period of time and include elements of host-pathogen interactions, epidemiology, genetics, as well as the development of treatment options. Only three years from its discovery in California, the entire genome of *Phytophthora ramorum* (Werres, de Cock, Man int Veld) was sequenced. The availability of the genome offers endless possibilities for research, and it has already been tapped to provide the strongest evidence yet in support of an exotic nature of this pathogen both in California forests and in European nurseries. Finally, this emergent disease highlights the inevi-

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table connectivity between the ornamental plant business and the health of native forests.

1994–1999

A disease without a cause.

The coastal forests of California are famous worldwide for the presence of a unique endemic tree, the redwood (*Sequoia sempervirens* [D. Don] Endlicher). This tree ranks as the tallest and among the largest in the world, a trait that has captured the imagination of tree lovers and timber companies alike. Costal forests of Northern California also include several additional timber-producing species such as Douglas-fir (*Pseudotsuga menziesii*) (Mirbel) Franco and Port Orford Cedar (*Calocedrus decurrens* [Torr.] Florin). It should not come as a surprise that when it was first reported in late 1994 that a previously unnoticed disease was killing non-marketable tanoak trees (*Lithocarpus densiflora* [Hook. & Arn.] Rehd.) (Svihra, 1999a), the local forestry community was not swept away (Fig. 1). Tanoaks have generally been seen as a weed species that creates difficulties for regenerating coniferous timber species. However, tanoak plays important ecological roles in these forests. Often the main tree species associated with redwoods, it is a major ectomycorrhizal host in the predominantly endomycorrhizal redwood forest; in a recent study 127 distinct ectomycorrhizal taxa were reported from a single site of pure tanoak (Kordesch *et al.*, 2005). It is undoubted that this mycorrhizal richness plays an important role in nutrient cycling in many coastal forests. Tanoaks are also known to support abundant wildlife, through acorn production and providing sites for nesting.

When local San Francisco Bay Area arborists and farm advisors noticed the disease had started to affect coast live oak (*Quercus agrifolia* Née) (Svihra, 1999b), a greater concern arose among pro-

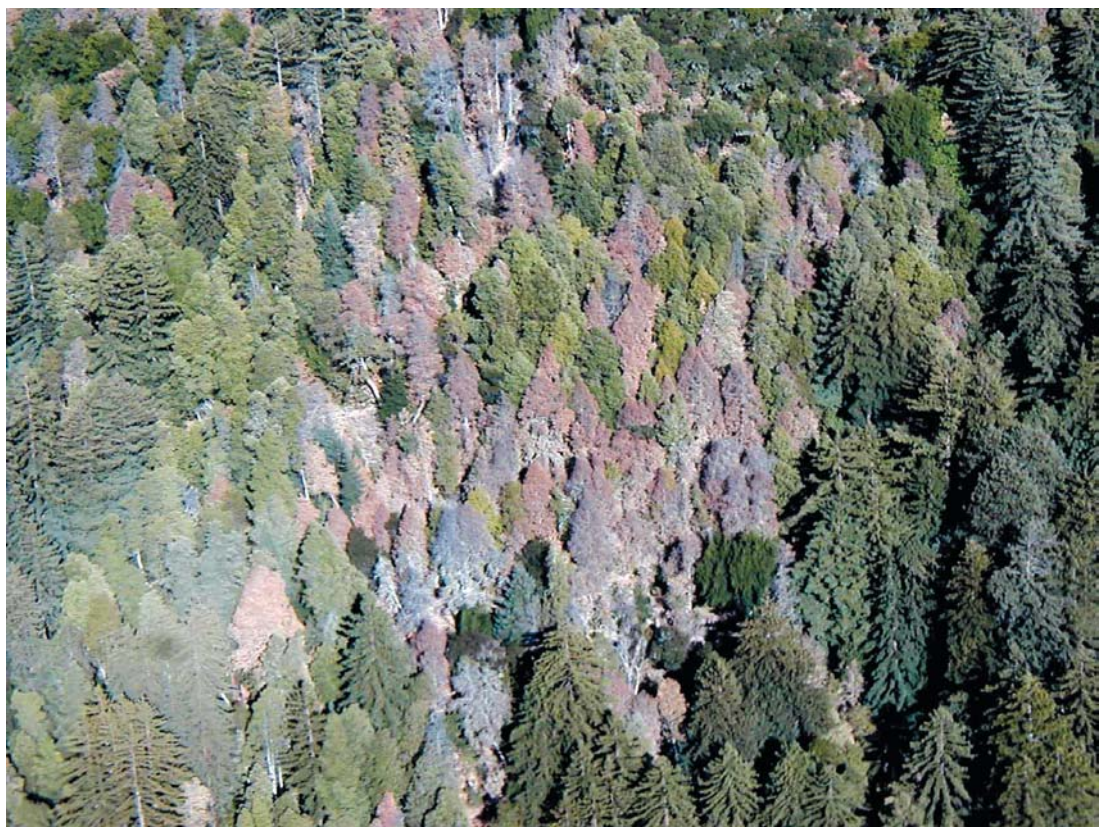

Fig. 1. Overstory mortality of tanoak (*Lithocarpus densiflorus*) caused by *Phytophthora ramorum* in Monterey Co. (Big Sur), CA, USA. (Photo by S. Frankel)

fessionals and the public. In order to understand the reason for the differential public reaction to the disease on oaks, one has to realize: 1) coast live oaks are one of the key drought tolerant and dominant tree species along the central and southern coast of California, 2) unlike tanoak, coast live oak often acts as a keynote tree in some habitats, 3) coast live oak trees are extremely abundant as a landscape tree throughout their range, and their contribution to property value is considerable in upscale Marin, Sonoma, Napa, San Mateo and Santa Cruz communities.

The newly-acquired visibility of the disease on oaks became enough of a public concern to catch the attention of politicians and administrators. In 1999, the first funds for research on the disease were made available by the USDA Forest Service and by the University of California. This led to the discovery of the causal agent of this new disease, called locally Sudden Oak Death. The common name was quickly picked up by the press and became a term of common knowledge and usage in the Bay Area by 2000. Trees affected by the disease showed a characteristic bleeding on the trunk; the bleeding would enlarge and affect the entire circumference of the tree without causing any visible symptoms on the tree crown (McPherson *et al*., 2002; Davidson *et al*., 2003b). Often a sunken area could be seen on the canker, and necrotic phloem and cambial tissue was observed following bark removal. Spherical, charcoal-like fruit bodies of *Hypoxylon thouarsianum* would often be produced in abundance on the main stem, and eventually bark and ambrosia beetles (*Pseudopityophthorus* and *Monarthrum* spp.) would attack the trunk. After these symptoms were first observed, the entire tree crown would brown over a period of a few weeks, and the tree then would appear to die suddenly. This is the source of the common name "sudden oak death". Subsequent research has found, however, that overall progression of symptoms culminating in tree death often takes a year to many years.

A closer look at the symptoms also revealed differences between oaks and tanoaks; while on oaks the bleeding affected adult trees and was mostly noticeable at the base of the trunk, all age classes of tanoaks had symptoms. Furthermore, bleeding cankers could be observed throughout main stem and branches of tanoaks.

But what was causing the bleeding cankers? Although consistently associated with dying trees, insects have typically been considered to be opportunistic colonizers of stressed trees. It seemed unlikely that they were the primary cause of the observed tree mortality, nonetheless in absence of a better candidate some researchers had temporarily indicated insects as a potential cause of the new disease (Svihra, 1999c). The bleeding was strikingly similar to symptoms associated with *Phytophthora* spp. on other tree species. But instead of coming up into the tree stem from the root system, as expected of a soilborne *Phytophthora* species, stem lesions clearly ended at the soil line or occurred solely on aerial plant parts. To add further confusion, no clear association between water and presence of infection could be shown at the landscape level. Infected trees were not found in draws or in areas subject to flooding, but they were spread across the landscape; in many areas, infected trees were first observed on ridges and crests.

2000

The discovery of the causal agent

To elucidate the cause of SOD, a dual approach to determine the causal agent of the bleeding cankers and an analysis to show that bleeding cankers were responsible for SOD (Garbelotto *et al*., 2001a, Rizzo *et al*., 2002). In June 2000, a *Phytophthora* species was consistently isolated from bleeding oaks and tanoaks at different locations. By the early fall, Koch's postulate had been completed on seedlings through artificial inoculations, and by the late fall of 2000, inoculation experiments on adult oaks and tanoaks were successfully completed (Rizzo *et al*., 2002). Inoculated trees developed large girdling stem cankers that started bleeding approximately a month after inoculation. Cankers were obviously caused by the inoculated *Phytophthora*, as *Hypoxylon* and beetles were only visible on these trees long after the lesions had girdled the stems. The second line of research was completed thanks to a large application of permethrines just recently completed by a professional arborist in a private ranch in Nicasio (Marin County) (Garbelotto *et al*., 2001). Researchers were able to outline two 1 ha plots on two different mountains in which all oak trees had been treated with insecticides. Adjacent to each of the two plots, two

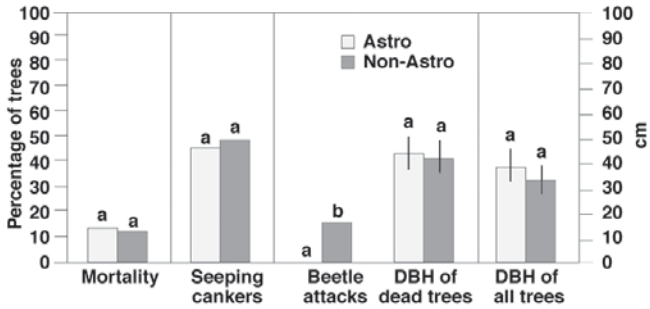

Fig. 2. Two adjacent sites in a forest stands have comparable oak populations as exemplified by the DBH (diameter at breast height). Each site contained 67 oaks. In the site treated with the permetrhine insecticide Astro, only beetle attacks were controlled, while mortality rate and bleeding caused by *Phytophthora ramorum* remained unchanged. These data showed beetles were not the causal agent of SOD. (Printed from California Agriculture 55(1), 9–18, 2001, by permission of the Regents of the University of California, CA, USA).

untreated plots of equal area and forest type were studied. The results showed that treated plots had no beetle attacks, but had the same number of bleeding cankers and the same number of dead trees as untreated plots, where beetle attacks were abundant. These results indicated that beetles were not the primary agents causing SOD, and corroborated the role played by the recently isolated *Phytophthora* species (Fig. 2). This finding led to the withdrawal of recommendations to treat the new disease with insecticides. The primary focus of research shifted to the newly discovered pathogen.

The discovery of a plausible cause for SOD allowed for the consolidation of efforts to fight this new threat to California coastal forests. Between August and October 2000, a consensus group consisting of public agencies, non profit organizations, and private interest groups was formed to address SOD in California in a comprehensive and coordinated manner. The group was known as the California Oak Mortality Task Force (COMTF). By January 2001, County, State, and Federal legislators and politicians supported funding SOD research and management by providing a combined amount of over 5.7 million US dollars in emergency funds. In January 2001, Oregon established the first quarantine on importation of wood, mostly firewood, from California.

Meanwhile, plant pathologists were attempting to determine which *Phytophthora* species they had isolated. Deciduous sporangia and large terminal or lateral chlamydospores were the trademark of the microbe growing in the Petri dishes in their laboratories, forming extremely knobby and "tormented" appressed colonies on corn meal agar. It was quickly realized that it was an undescribed species of *Phytophthora*. DNA sequence analysis revealed this new species to be a close relative of another putatively exotic *Phytophthora* present in Northern California and Oregon, *P. lateralis*. *P. lateralis* was first observed in Oregon nurseries in the first half of the 20th century, but it soon escaped the controlled environment of nurseries causing destruction in Port Orford Cedar populations in Southern Oregon and then Northern California (Hansen *et al*., 2000). With the exception of a few infections of pacific yew (*Taxus brevifolia*), *P. lateralis* is known to only affect Port Orford cedars. Its epidemiology is that of a typical waterborne and soilborne *Phytophthora* species, causing infections of roots along waterways and dirt roads. Such differences between the two species in terms of hosts and biology were not necessarily surprising, given that the DNA sequence homology between the two species was high (97%) but different enough to represent two clearly distinct evolutionary units. Although it may be tempting to think of this phylogenetic knowledge as a curiosity without practical consequences, the early genetic work on this pathogen had many unpredictable uses and consequences. At the time of the discovery of the new *Phytophthora*, a paper by Cooke *et al*. (2000) had just been published presenting a comprehensive phylogeny of the genus *Phytophthora* based on the ITS (internal transcribed spacer) region of the rDNA (ribosomal DNA) operon. The work showed that ITS sequence could differentiate most *Phytophthora* species and provided a functional database for the molecular identification of species belonging to this genus. Using this database the relationship between *P. lateralis* and the SOD *Phytophthora* was discovered.

In the Fall of 2000, British plant pathologist Clive Brasier heard a description of a new and unnamed *Phytophthora* species in the Netherlands, he noticed a striking similarity with the cultures he had been shown in California just weeks before. Researchers from California and Europe,

quickly exchanged ITS sequences. The sequences from isolates collected in Germany and The Netherlands perfectly matched those of isolates from California. A connection between the two continents had been made, only to be broken and then made again in the months to follow. The most striking fact about the presence of the same organism in Northern Europe, an area with little ecological or evolutionary similarities with California, was that in Europe it was known exclusively as a pathogen of ornamental *Rhododendron* and *Viburnum* spp. (arrowood) causing a leaf blight and a branch dieback in the first group of hosts and a root collar canker in the second. Based on this information, California researchers were able to find infection caused by the SOD pathogen on rhododendrons in nurseries in California. Symptoms on rhododendrons were identical to those described in a paper written in German for professional growers (Werres and Marwitz, 1997), the morphology was identical to that of the SOD *Phytophthora,* and by January 2001 it had been confirmed that the ITS DNA sequence of isolates obtained from rhododendrons was identical to that of isolates obtained from oaks.

Although it was in California that the importance of the pathogen had been discovered, the European group had isolated the same organism in 1994, years before the American scientists. The burden of describing the new species fell on the European group who named it *Phytophthora ramorum*, or "of the branches", to highlight the dramatic dieback symptoms it causes on *Rhododendron* spp. (Werres *et al*., 2001). The fact that symptoms on ornamental plants were mostly aerial was in agreement with the observation that symptoms on oaks and tanoaks were also aerial.

2001–2002

The host and geographic range of *P. ramorum* expands.

With the added knowledge that the pathogen could cause leaf necrosis and twig dieback on plants completely unrelated to oaks, California researchers ventured back into the forests, sampling every unusually symptomatic plant they could encounter. The approach was to process each sample twice: half of the symptomatic portion of each sample was cultured on a *Phytophthora* selective medium, and the other half was processed for DNA analysis using an experimental *P. ramorum*- specific PCR-based nested diagnostic assay (Hayden *et al*., 2004). In the period between January 2001 and September 2002, this combined approach resulted in the discovery of 10 new hosts for *P. ramorum* and 23 new infested areas in four California counties. Many of these findings were first determined using the PCR diagnostic and later confirmed by traditional isolation methods (Garbelotto *et al*., 2003). Data compiled during the California *P. ramorum* survey, highlighted the importance of molecular pathogen detection but also indicated that success of DNA-based detection rarely approached 100%. Detection success was highly dependent on plant substrate, plant species, and time of year. Best detection was achieved in the late spring-early summer and with the detection rate gradually decreased during the remainder of the year. This was the first time that host and geographic ranges of a new fungal pathogen had been discovered with the use of a PCR-based assay (Garbelotto, 2004).

By September 2002, recognized disease symptoms in North America forests included stem and branch cankers of oaks, tanoaks, black oaks, and Shreve's oaks; cane cankers and leaf abscission on huckleberry (*Vaccinium ovatum*) and manzanita; leaf spots or blotches on California bay laurel leaves (*Umbellularia californica*), buckeyes (*Aesculus californica* [Spach.] Nutt.), and honeysuckle (*Lonicera* spp.); angular spots on toyon (*Heteromeles arbutifolia* [Lindl.] M. Roemer) and coffeeberry (*Rhamnus* spp.); marginal scorch of maple leaves (*Acer macrophyllum* Pursh), leaf blotch and branch dieback of Pacific madrone (*Arbutus menziesii* Pursh) and rhododensdrons; tip wilting in Douglas-fir; needle necrosis and dieback of lower branches or of epicormic shoots in redwoods (Fig. 3) (Garbelotto *et al*. 2003 and references within). The pathogen could be isolated from above ground parts of all official hosts but one (*Actrostaphylos* spp.) with varying success frequencies, depending on host species, substrate and season; and it could be baited mid-winter to early fall from soil and year round from water streams (Davidson *et al*., 2003).

The natural spread of SOD driven by plants other than oaks

The ability to sporulate on each host will determine the role played by that host in the epidemiology of the disease. It was the serendipitous

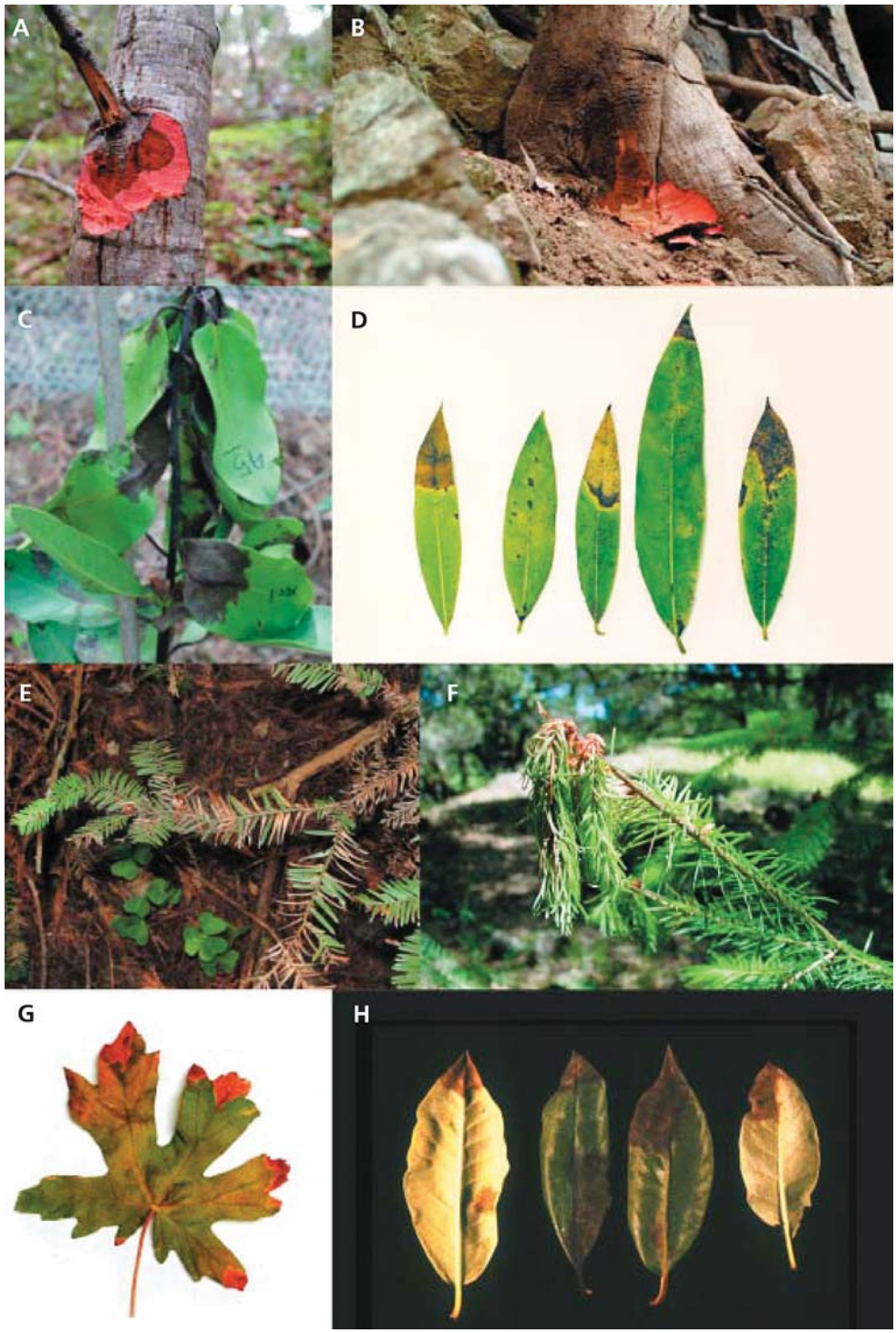

Fig. 3. Symptoms caused by *Phytopthora ramorum* on a range of hosts in California: A, aerial stem canker on tanoak; B, basal canker on tanoak ends at soil line; C, leaf blight and twig canker on Pacific Madrone seedlings; D, necrotic lesions on bay laurel leaves; E, needle blight on redwood; F, apical wilting on Douglas-fir; G, scorch on big leaf maple; H, leaf blight on rhododendron leave. (Printed from California Agriculture 57(1), 18–23, 2003, by permission of the Regents of the University of California, CA, USA).

discovery of infection on one of the hosts that held the key to understand the biology and the epidemiology of the disease in the forest. In May 2001, necrotic bay laurel leaves were included as a "false" positive to test the validity of the newly developed *P. ramorum* PCR assay. Not only did the assay come back positive, but the intensity of the PCR signal was much stronger than what seen for any other host. Bay laurel was soon determined to be the key species infected, although in a non-lethal way, by *P. ramorum* in California. Not only was bay laurel the most commonly *P. ramorum* infected tree, but sporangia production was found to be very abundant on bay leaves. This finding provided a clue regarding the source of the aerial inoculum. It was determined that the presence or absence of sporangia in rainwater was correlated with the proximity of infected bay laurel trees, but not to the presence of infected oak trees. All of a sudden, the oak-centric vision of Sudden Oak Death gave way to a much more complex pathogen life cycle. Further research confirmed that sporulation on oak wood hardly ever occurs, thus oak species can be regarded as non-contagious dead-ends in the life cycle of the pathogen (Davidson *et al*., 2003; Davidson *et al*., in press). Sporulation has subsequently been found to be very abundant on bay laurel leaves and tanoak twigs, and present but at significantly lower levels on redwood needles. The role played by hosts other than the ones mentioned above, as well as by infested soil and water in the natural spread of the disease is still not clear (Rizzo and Garbelotto, 2003; Davidson *et al*., 2005).

2002–2003

SOD as a public issue

Probably 2002 was the year in which SOD attained the highest visibility in the popular and scientific press, as well as among politicians and the general public. In order to understand such popularity, we need to step back and look at the unfolding of events since the onset of the disease and highlight their relevance in terms of public impact. The riddle of the mysterious disease had been disturbing for California residents over a period of several years; its resolution, at least by understanding the cause of such a disease, combined with the finding that the culprit was a new microbial species, caught the imagination of audiences worldwide. In 2000 and 2001, dead trees were common and extremely visible, similar to a wildfire spreading throughout the landscape. The infested area also coincided with one of the most populated and visited areas in North America, the San Francisco Bay Area (Fig. 4). Contrast this with a pathogen such as *Phytophthora lateralis*, whose geographic range remains confined to a less densely populated and relatively little visited area of the country. The international dimension of the problem, as exemplified by the potential connection between the West Coast of the USA and Europe, and the "interdisciplinary" element of the disease, connecting a disease in the wild and the ornamental plant business, further added to the lure of the subject. Last, but not least, the discovery of the pathogen started a cascade of discoveries. In light of the urgency to stop the disease from

Fig. 4. Distribution of confirmed cases of *Phytophthora ramorum* in forests of California and Oregon in January 2005. (Courtesy of M. Kelly, U.C. Berkeley, CA, USA).

further spreading, discoveries pertinent to the pathogen's range and epidemiology were officially released by University and Government media offices. The media coverage provided an exceptional outreach tool that not only educated the public about SOD, but also arose the awareness about the potential impact of exotic microbes on native ecosystems. The downside of such extensive media coverage was the impossibility to control the quality and the truthfulness of all information published. For instance, the news of redwood as a new host for *P. ramorum* were released prematurely without the input and the consent of the researchers involved, University, or Government media offices, leading to erroneous interpretations of the potential effects of the pathogen on this plant host. Depicting a "sudden redwood death" scenario, the news circulated around the globe, while in reality the pathogen was being investigated for causing needle lesions of no consequence for the health of the infected trees.

The basic ecology of *P. ramorum*

The zone of infestation at the end of 2002, as the current one in 2005 (Fig. 4), comprised a fragmented distribution of the disease in close (30 km) proximity of the Pacific Ocean or of the San Francisco Bay. The general frequency of reported infestations decreased as one moved away from the Bay Area in either northern or southern directions. The distribution of the disease, centered not on any climatic or habitat distribution, but on an anthropogenic factor (urban metropolitan area), combined with the aggressive behavior of the pathogen on tanoaks and oaks, suggestive of lack of co-evolution between host and pathogen, were the two main factors cited in support of an exotic nature of *Phytophthora ramorum* (Rizzo and Garbelotto, 2003). The patchiness of the disease could have been explained by a variety of reasons, but two of them appeared as fundamental: 1) the habitats favorable to the disease were extremely fragmented for natural or historical reasons, 2) the pathogen was not yet in equilibrium, and had not occupied its entire niche because it was not native but of relatively recent arrival. By the end of 2002, several studies began to define the climatic parameters of the pathogen and host-habitat associations (Davidson *et al*., 2002; Kelly and Meetenmeyer, 2002; Swiecki and Bernhardt, 2002; Garbelotto *et al*., 2003).

In 2002, it became clear that, with few exceptions, all infections by *P. ramorum* in the wild were correlated with proximity to closed canopy woods of two broad types: mixed evergreen forest dominated by coast live oak and the redwood- tanoak forests, also characterized by the presence of Douglas-fir (Rizzo *et al*., 2005). The presence of potential hosts outside of these habitats does not seems to represent an available niche for the pathogen as exemplified by the fact that infections have not been found on coast live oak trees in more open and drier savannah type habitats are (Rizzo *et al*., 2002b). In 2002, the survey of *P. ramorum* in California had finally reached a sufficiently large size to make inferences based on its results. It began to emerge that in California, disease sites were characterized by the presence of bay laurel, tanoak, or both (Rizzo and Garbelotto, 2003). Just prior to the finding of *P. ramorum* on bay laurel, a study determined that the presence of bay laurel was one of the few variables that showed a strong positive correlation with the presence of disease (Swiecki and Bernhardt, 2002). Another study confirmed the clustered nature of the disease previously observed by field researchers (Kelly and Meentemeyer, 2002).

By the end of 2002, the distribution of the pathogen included not only the California zone of infestation, but also a relatively small area in Southern Oregon (discovered in 2001), and more than 150 nurseries in many EU countries. New species including the first herbaceous host, Pacific starflower, (Hüberli *et al*., 2003) were added to the expanding list of hosts and potential hosts (Table 1), and quarantine and inspection regulations are active in many countries including the USA, the UK, Canada, Australia, South Korea, and the EU. Oregon began an eradication program that included cutting and burning infected plants at a handful of small sites in Curry County (Goheen *et al*., 2002).

Funding for SOD research and management continued at the State and Federal levels, now also joined by three California-based Private Foundations. In 2002, the US Department of Energy announced it had selected *P. ramorum* for the sequencing of the entire genome, an interesting choice considering its recent discovery and the limited biological genetic information available on this pathogen.

Table 1. Plant hosts susceptible to *Phytophthora ramorum* in western North America, including type of symptoms described for each host and recorded disease severity.

Host genera	Symptoms[a] type	Disease[b] severity
Acer	1	1
Arctostaphylos	2	2. 3
Aesculus	1	1
Camellia	1. 2	2. 3
Clintonia	1	2
Corylus	1	1
Dryopteris	1. 2	1. 2. 3
Heteromeles	1.2	2. 3
Lithocarpus	1. 2.3	2. 3. 4
Lonicera	1	1. 2
Pieris	1. 2	2. 3
Quercus	3	4
Rhamnus	1. 2	
Rhododendron	1. 2	2. 3
Rosa	1	1
Rubus	1	1
Sequoia	1. 2	1
Sambucus	1. 2	2. 3
Smilacina	1	1
Taxus	1	1
Tozicodendron	3	3
Trientalis	1	1. 3
Umbellularia	1	1
Vaccinium	1. 2	2. 3
Arbutus	1. 2	2. 3

[a] Symptoms type: 1, foliar symptoms; 2, twig or branch dieback; 3, stem canker; 4, root collar canker.

[b] Disease severity: 1, only foliar; 2, leaves and branch dieback; 3, plant mortality; 4, extensive plant mortality.

The discovery of other new aerial *Phytophthora* species

By the end of 2002 it was clear that *P. ramorum* was only one of several aerial *Phytophthora* spp. present in the forests of California. At least five different taxa were found causing similar symptoms on many of the same hosts infected by *P. ramorum*. While three were rare, two species were found in relative high abundance particularly on bay laurel, coast live oak, and tanoak. The geographic distribution of these species seemed to be more extensive that that of *P. ramorum*, suggesting either they were the result of earlier introductions, or native. Both species were homothallic, and appeared to be less pathogenic than *P. ramorum*. The least pathogenic of the two species was formally described in North America and called *P. nemorosa* ("of the woods") (Hansen *et al*., 2003). The other species was found to be an identical match to a species just described in Europe and called *P. pseudosyringae* (Jung *et al*., 2003). Interestingly, this species was described as a root pathogen in Europe, while in North America, aerial symptoms appear to dominate. Both species are close relative to a *Phytophthora* specialized on English Holly (*P. ilicis*). Aerial Phytophthoras are obviously frequently encountered on the West Coast of the USA, and may have been unnoticed elsewhere in the world.

2003

***Phytophthora ramorum* in the nurseries: differences and similarities between Europe and North America**

In 2003, *P. ramorum* in commercial nurseries took the front stage. The host list of ornamental plants increased significantly, and so did the number of European countries with infested nurseries. Nurseries also became important players in the North American SOD saga. In May 2003, *P. ramorum* was found in California and Oregon nurseries well outside of where the pathogen was established in coastal forests, making it apparent that these nursery infections did not originate from infested surroundings but had been acquired through the trade of infected ornamental plants.

In June 2003, nurseries in Washington State and British Columbia (Canada) were reported to be infested. In August 2003, it was confirmed that the European A1 mating type was present in some Oregon (Hansen *et al*., 2003b), Washington and British Columbia nurseries, often in proximity of plants infected by the A2 mating type (Garbelotto, personal unpublished data). These findings are relevant to understanding the possible mode of introduction of *P. ramorum* and may ultimately help researchers determine the area of origin for *P. ramorum*.

Since 2001, it had been suggested that significant phenotypic (e.g., colony morphology and stability, host × pathogen interaction, growth rates, mating type) and genotypic (e.g., mitochondrial sequence polymorphisms, Amplified Fragment

Length Polymorphism profiles, genetic structure) differences distinguished the European from the North American populations (Garbelotto *et al*., 2002b; Brasier, 2003). By 2002, it was confirmed that all forest isolates tested from North America were of the A2 mating type, while isolates from nurseries in Europe belonged to the A1 mating type (Brasier and Kirk, 2004). With the exception of a single A2 isolate later found in Europe (Werres and De Merlier, 2003), this split remains true in 2005. In June 2001, the first report comparing US and EU isolates with the use of AFLPs was presented (Garbelotto *et al*., 2002b); although the sample size was relatively small, the US-EU split was also convincing based on this dataset. A single nucleotide polymorphism alternatively fixed in the two populations allowed for the development of an RFLP diagnostic test able to differentiate individuals from the two continents (Kroon *et al*., 2004). A more comprehensive AFLP study provided information on the genetic structure of the pathogen within and between continents (Ivors *et al*., 2004). In North America a single genotype was recovered at all sites about 75% of the time. At some, but not all locations, single isolates of other very closely related genotypes (95% similarity index) were found. The European clade was found to be more complex, with subclades at 90% similarity index, each containing very closely related individuals. Isolation of identical genotypes occurred infrequently, and in each case the identical genotype was isolated only twice. Multilocus analysis indicated the European and US lineages were clearly distinct and their genetic structure unlikely to be the result of recent sexual reproduction; however sexual reproduction could not be excluded within continents. Because of the presence of both mating types in Europe, although in an extremely skewed proportion, sexual reproduction could be a possibility. Despite the fact that all US forest isolates belonged to the North American clade and were mating type A2, isolates from nurseries in the Pacific Northwest region of the US were of both mating types and fell into both AFLP clades (Ivors *et al*., 2004)

Results from all available lines of evidence showed the European and North American epidemics to be characterized by the limited genetic variability that is the trademark of exotic pathogens and pests. Comparative analysis of populations from the two continents indicated they each represented distinct lineages that must have generated through significant genetic isolation. The implications of this discovery were that the original introduction of *P. ramorum* in California probably did not come from Europe. Nonetheless, the later finding of A1 isolates belonging to the European AFLP clade suggested that the trade of ornamental plants may be responsible for long intercontinental spread of this pathogen.

The presence of A1 mating type individuals in North American nurseries was immediately seen as problematic from a regulatory standpoint. Not only were A1 nursery isolates potential mates for A2 individuals, allowing for recombination, but they seemed to be characterized by sufficient phenotypic differences to potentially cause a different disease if they were to escape to the wild. In 2003 the first news of tree infection by *P. ramorum* outside of nurseries in Europe came from the Netherlands and the United Kingdom (Brasier *et al*., 2004). While in both cases the connection with infested nurseries was not only plausible but probable, the Dutch wild infestation was directly linked to earlier plantings of rhododendrons coming from an infested nursery. These events emphasized the need for strict controls and regulations in the ornamental trade.

The first treatments for the control of SOD.

In October 2003, the California Department of Pesticide and Regulations accepted the registration of two products for the treatment of Sudden Oak Death (Anonymous, 2003). The registration filed by the manufacturing company was supported by a series of controlled experiments both *in vitro* and *in planta* (Garbelotto, 2003). Studies had started in September 2000 and were based on the experience of forest pathologists in Western Australia and Avocado growers worldwide that were treating *Phytophthora cinnamomi* infections with phosphite (Guest *et al*., 1995, Hardy, 1999). By March 2001, the first results were obtained *in planta*, and the first publication came out in June of the same year (Garbelotto *et al*., 2002). Although the mode of action of phosphonic acid, the active component in phosphite, is still not completely understood, it is known to trigger the production of secondary metabolites, plant products known to have strong antimicrobial properties (Jackson

et al., 2000). This mode of action hinges on affecting the physiology of the tree with limited side effects on microbial communities and as a result phosphite treatments are environmentally friendly and appropriate for uses in forestry. The newly registered treatments were exclusively for the most susceptible hosts, oaks and tanoaks, as no significant research had been done on the other hosts. Preventive treatments using Agri-Fos were the most effective, and they appeared to cause a 50–100% reduction in growth rate of the pathogen on 80–90% of treated individuals (Garbelotto *et al*., 2002; Garbelotto, 2003). Soil drenches, foliar sprays, bark applications and injections were tested. Soil drenches and bark applications had no effects, foliar sprays were moderately effective but also caused significant phytotoxicity. Positive pressure injections (i.e., constant pressure is present until the entire amount of product is delivered) in the outer xylem were the most effective registration of the first product (Agri-Fos, Agrichem Manufacturing Pty. Ltd. Loganholme, Queensland, Australia). was essentially a label expansion to include a new pathogen and a new host. The second product registered for treatment of sudden oak death (PentraBark, Agrichem Manufacturing Pty. Ltd. Loganholme, Kld, Australia) was an organosilicate surfactant that allowed for pentration of the phosphite without injections (Garbelotto, 2003). As opposed to injections, which required skilled applicators and are generally recommended only for trees with a completely sound trunks, this treatment did not damage the tree. Although originally bark treatments were recommended only for oaks, research in 2004 has shown their efficacy on tanoaks as well (Garbelotto, personal communication).

Other treatments options started to become available as data supporting their efficacy was provided through experimental testing. A 12-hour application of heat and vacuum completely inhibited *P. ramorum* growth in infected bay leaves to be used as a spice, without affecting the leaves' taste (Harnik *et al*., 2004). Composting following the United States Environmental and Protection Agency (EPA) guidelines for commercial composting in California ensured a complete inhibition of sporulation and growth by the pathogen and suggested that composting represents a viable sanitation practice. Flash-heat treatments of 30 minutes at 55°C were not effective, and in the presence of chlamydospores a minimum of 2-week exposure at 55°C was needed to effectively affect the viability of the pathogen (Garbelotto, 2003b).

2004

Infections in new hosts, areas, and nurseries indicate *P. ramorum* is a persistent and complex pathogen.

The year 2004 represents an ideal place to end a chronological review regarding early research on *P. ramorum*. New hosts discovered in forest settings were extremely varied and included the first monocotyledons (*Smilacina racemosa* and *Clintonia andrewsiana*) (Hüberli *et al*., 2005) the first pteridophyte (the fern *Dryopteris arguta*), the conifer Pacific yew (*Taxus brevifolia*), and finally, the wild wood rose (*Rosa gymnocarpa*) (Hüberli *et al*., 2004) which was also found to support sporulation by the pathogen. New infestations were discovered both at the edges of the zone of infestation and within counties already classified as infested, indicating an expansion of the areas colonized by the microbe.

In April, infestations were detected in two large Southern California nurseries. Symptom development was apparently very fast and linked to a single flooding event that affected the nursery after some unusually intense precipitation (Tjosvold, personal communication). This large infestation exemplified the difficulty in understanding disease etiology and development in the highly artificial environment of the nursery, where cultural practices and chemical treatments may at times have a highly fungistatic effect, thus masking disease symptoms. As a consequence of the infestation in these two California nurseries and of a nursery in Oregon, shipment of putatively asymptomatic plants resulted in infestation of 140 nurseries in 18 states within the USA, including California, Oregon, and Washington already known to have infested nurseries. At the same time, experimental evidence was found that rhododendron roots in pots could be infected by the pathogen (Jennifer Parke, personal communication), and that the pathogen could survive a long time in infected plant material buried in the potting mix, thus complicating disease diagnosis and control in the nursery setting (Shishkoff and Tooley, 2004).

The use of DNA-based diagnostics emphasized in new regulations

Acknowledging that diagnosis solely based on isolations was problematic, APHIS (Animal and Plant Health Inspection Services) an important branch of the USDA (United States Department of Agriculture) approved a diagnostic protocol in which ELISA and PCR-assay negatives were considered as valid, while negative isolations were not. On the other hand, positive isolations and positive PCR-assays were both recognized as valid to diagnose the presence of the pathogen. This regulatory decision acknowledged the difficulty of isolations of this pathogen, especially in light of the huge variability found among its many hosts and substrates, and takes advantage of published and validated molecular methods to diagnose the pathogen. By 2004, several methods were already published, and several were in press (Hayden *et al*., 2004; Kroon *et al*., 2004; Martin *et al*.; 2004; Bilodeau *et al*., 2005; Hughes *et al*., 2005). Quantitative PCR protocols also allowed for the determination of viability of *P. ramorum* in infected tissue. Although these viability indicators needed to be tested and may have been used only in special situations, e.g. in the absence of resting propagules, they still represented a step forward in the use of molecular techniques. A paper by Hayden *et al*. (2004) provided some realistic figures on success rates of detection of *P. ramorum* by PCR, showing a significant difference between detection success on the easiest substrate in the most favorable season (76% from bay laurel leaves in the spring) and for instance, the success rate from wood, again in the most favorable season (52%). Nonetheless PCR was always superior to isolations, independently of time of sampling or substrate.

Towards an understanding of the complex ecology of *P. ramorum* and of the effects of SOD on native forests.

Complex research studies presented in 2004–2005 showed sporulation to be linked to rainfall, and the lag phase between rainfalls and actual sporulation events appeared to be longer in the oak-bay evergreen forest than in the tanoak-redwood stands. Sporangia were collected up to ten meters from a source of infection, providing a scale for tree-to-tree contagion (Davidson *et al*., 2005), and data on survival of the pathogen in infected plant tissue in soil forest ecosystems, suggested that the pathogen can oversummer in well shaded leaves in the litter (Fichtner, 2005).

The first study looking at interaction effects between two pathogens, *P. ramorum* and *Botryosphaeria dothidea*, on Pacific madrone was completed, indicating a synergistic effect between the two, and defining *P. ramorum* as a clear primary pathogen on this species (Maloney *et al*., 2004). The relationship between infections by *P. ramorum* on oaks and secondary decay agents or wood boring insects was also investigated. Results showed that trees infected by *P. ramorum* have a greater likelihood to be colonized by secondary fungi and insects. The effect of this activity by secondary agents is tree failure, and as a consequence, a greater incidence of tree failures was detected when SOD was present in an area (Swiecki *et al*., 2005).

Information was also presented regarding pathogen effects on forest structure and composition (Brown and Allen-Diaz, 2005) and related changes in the resident fauna. For instance, two bird species were found to nest/feed on oaks and to avoid bay trees: these species obviously are significantly affected by SOD (Apigian and Allen-Diaz, 2005). When the epidemic started, it became apparent that a lot of the baseline information on the broad ecology of these forests was not well understood. This was even more evident for our understanding of the microbial ecology of soil organisms. Because of the severity of the disease on tanoaks, studies were initiated to study the mycorrhizal associations with this tree species. In a single stand where the only ectomycorrhizal; host was tanoak, an incredibly rich mycorrhizal community was described, comprising over 120 taxa (Kordesh *et al*., 2005). Further results indicated that physiological changes similar to these caused by a *Phytophthora* canker were differentially affecting the survival of different mycorrhizal taxa (Bergemann *et al*., 2005).

The final outcome of complex interactions such as those mentioned above will determine the overall impact the pathogen is going to have on the native plant species that make up these newly invaded ecosystems. Furthermore, disease severity is going to be mediated by environmental conditions, by the pathogen adaptability and genetics, and by the genetic structure and plasticity in the hosts species. While research on the pathogen and on interactions between the pathogen and the environment started right after the discovery of *P.*

ramorum in California, research on the host-end was more recent. By 2005 it appeared that in bays, coast live oaks, and tanoaks variable susceptibility levels existed among individuals. In the case of bay laurels, the available data suggested there may be significant differences also among populations. The experimental data, based on inoculations of detached branch cuttings (oaks) (Dodd *et al*., 2005), detached leaves (bays) (Hüberli *et al*., 2005b), and seedlings (tanoaks) (Hayden and Garbelotto, 2005) also suggested multiple genes to be involved in the regulation of the observed range of susceptibility. Overall heritability of this trait and whether reduced susceptibility is sufficient to withstand repeated exposure to the pathogen, had yet to be studied.

The genome of *P. ramorum* sequenced: implications for research and control

In June 2004, the Joint Genome Institute of the US Department of Energy in collaboration with the Virginia Bioinformatics Institute announced the completion of the sequencing of the genomes of *P. ramorum* and of the related *P. sojae*, a pathogen of soy bean (http://genome.jgi-psf.org/ramorum). This event marked the fastest sequencing of the entire genome of an organism since its discovery, only four years (considering July 2000 as the date of discovery of the organism), and provided an endless source of information for studies at the genetic and genomic level. The first application of the genome sequence was the development of microsatellite loci to be used for the analysis of the genetic structure and relatedness of the European and North American populations (Ivors *et al*., 2005). The study revealed ample gene duplication within the genome of *P. ramorum* and confirmed the US infestation to be derived from a single genotype, while a few closely related genotypes could be detected in Europe. European and North American populations can be easily differentiated as genetically isolated from one another with approximately 80% of the alleles alternatively fixed. The limited genetic variation in both continents, and especially in the US, provided the strongest data in support of the exotic nature of the pathogen. The microsatellite data not only corroborated the results provided by the previous work on genetic structure of *P. ramorum* using AFLPs, but indicated the limited AFLP variation was due to recent post-introduction events such as mutations or mitotic recombination, and not to the introduction of different closely related genotypes (Garbelotto *et al*., 2004b). An unexpected result of the microsatellite analysis was the discovery of one isolate bearing alleles typical of both continents. This isolate had a combination of characteristics that is unique among *P. ramorum* isolates and indicated the presence of a third lineage, where recombination between the two originally described lineages (the US and European ones) had occurred in the past or was still occurring at the time the analysis was performed. The discovery of this new isolate brought researchers a step closer to the discovery of the origin of this pathogen and provided evidence that the actual *P. ramorum* epidemics are caused by individuals derived from a sexual outcrossing population (Garbelotto *et al*., 2005).

Final remarks: the unique biology of *P. ramorum* and the need to place research results within the historical context.

The first four years of the history of research on *P. ramorum* in California are summarized in this article. Most forest diseases introduced in recent times have been characterized by a limited host range. Other exotic pathogens with a broad host range are generally characterized by a homogeneity in types of symptoms and in mode of actions (e.g. they affect the root systems of all hosts). *P. ramorum* poses a new challenge to researchers: as it is a pathogen of native and ornamental plants alike; causing girdling stem cankers on oaks and tanoaks while its infection on a wide and separate list of hosts simply results in foliar blotches and twig dieback. Its natural epidemiology is complex and unique among plant pathogens: oaks are lethally affected by the disease but they are not contagious and represent dead-ends for the spread of the pathogen; bay laurels are relatively asymptomatic carriers of the disease, with symptoms limited to black lesions on portions of their leaves. Abundant sporulation by *P. ramorum* can be found on these lesions, which have limited impact on the health of bay trees themselves but will cause the lethal stem infections on oaks. The consequences of this differential host role on the evolutionary trajectory of this plant pathogen are unknown and unpredictable, maybe with more similarities to patterns of some animal diseases than to other plant

pathogens. This epidemiological cycle of SOD in fact, resembles those of animal diseases such as malaria or West-Nile virus.

While at best we can hope to slow down its spread at the local level, thanks to the implementation of so-called "eradication" efforts, the best approach is to prevent either successive introductions of different isolates of the same pathogen in the same area, or any introduction in new areas. Recent still ongoing studies on *P. ramorum* show an amazing level of phenotypic plasticity even within identical genotypes, and the genetic work in the laboratory has shown the potential for sexual recombination is real. Hence, both movement of genotypes from one infested area into another, and introductions into new areas should be avoided. Most evidence, in fact, seems to suggest this pathogen has a relatively limited ability to move even in the medium range of distances. If this limited ability of the pathogen to move is supported by further research, the prevention of local introductions combined with an early detection will be an essential aspect of disease management. While introductions in the past were often unknown and hence went undetected, we have the advantage of being alerted about possible introductions of *P. ramorum* and there is a good selection of available diagnostic assays.

The presence of the disease around the San Francisco Bay Area, is probably not the result of unique ecological conditions, but the outcome of an introduction event that occurred in the Bay Area. Until the time of writing of this paper, all natural infestations have occurred within a relatively narrow coastal strip, approximately always within 20 miles from either the Pacific Ocean, or the San Francisco Bay. One of the striking features of the entire infested region is the presence of persistent thick fog during the dry season. Is the link with fog an absolute one for natural spread of the disease or is it simply explained by the history of its introduction? The presence of fog, combined with years of abundant rainfalls, may be one of the factors that has allowed *P. ramorum* infestations to reach epidemic levels on the coast of California, but the pathogen has the ability to spread well beyond the fog belt. The type of disease it may cause outside the fog belt may be quite different from what has been described for the present-day zone of infestation, characterized maybe by a much shorter period of activity for the pathogen, symptoms may be less dramatic and present exclusively in those years when conditions are extremely conducive for the spread of pathogen.

In the case of an introduced microbe, such as *P. ramorum*, it is not only the interaction of host, pathogen, and environment that will determine the type and intensity of disease development, but the historical component will also play an important role. This role may be particularly significant in the early phases of disease spread; when the exotic pathogen has not yet reached equilibrium in its new environment. Thus, results of investigations on host and geographic range of an introduced microbe should be interpreted with caution, in light of the potential confounding effects due to the introduction events themselves.

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