The Threat of Hybrid *Phytophthoras*

Janna Beckerman, Stephen Goodwin and Kevin Gibson

Janna Beckerman, Associate Professor, Department of Botany and Plant Pathology, Purdue University. West Lafayette, IN 47907; E-mail: jbeckerm@purdue.edu

Stephen Goodwin, Professor, Department of Botany and Plant Pathology, Purdue University. West Lafayette, IN 47907; E-mail: sgoodwin@purdue.edu

Kevin Gibson, Associate Professor, Department of Botany and Plant Pathology, Purdue University. West Lafayette, IN 47907; E-mail: kgibson@purdue.edu

Beckerman J, Goodwin S, Gibson K. 2014. The Threat of Hybrid *Phytophthoras.* In: Wilkinson KM, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2013. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-72. 12-17. Available at: http://www.fs.fed.us/rm/pubs/rmrs_p072.html

Abstract: The majority of invasive plant pathogens have resulted from the introduction of exotic organisms. However, another mechanism for invasiveness results from hybridization between species. This phenomenon has been documented in plants and animals, but its role in plant pathology has only recently been recognized. With more than 100 species of *Phytophthora* identified to date, and little information regarding their biogeography and native habitat, *Phytophthora* hybrids are difficult to detect. Unfortunately, recent taxonomic surveys for *Phytophthora* in the nursery, greenhouse and landscape have identified multiple hybrids involving different parental species. Their spread via the international plant trade poses significant risks to ecosystems throughout the world.

Key Words: hybrid plant pathogens, transgressive segregation, invasive plant pathogens

Introduction

Plant disease epidemics that result from the introduction of exotic organisms are well-recorded phenomena (Brasier 2008). First documented with chestnut blight, and later, Dutch elm disease, Jarrah decline, and sudden oak death (SOD), diseases caused by introduced plant pathogens have changed ecosystems. In the case of Jarrah decline and SOD, *Phytophthora* species were the causal agents. Unlike other plant disease epidemics, these two introduced *Phytophthora* species, *P. cinnamomi* and *P. ramorum*, have broad host ranges, and are capable of infecting hundreds of species of plants. Although the spread of *P. cinnamomi* began in colonial times by unknown means, the primary spread of *P. ramorum* was through the nursery industry (Frankel 2008).

Unlike true fungi, which are members of the Kingdom Fungi, *Phytophthora* species are members of the Kingdom Stramenopila, which includes everything from large 100-ft (30-m) seaweeds (phaeophytes or brown algae) to diatoms, golden algae, and water molds (Phylum: Oomycota). Members of the oomycota, often called oomycetes, are diploid (unlike most true fungi, which are haploid) and their cell walls contain cellulose, whereas those of true fungi are composed primarily of chitin. Oomycetes are fungus-like in that they produce hyphae to invade and colonize a plant, and possess a life cycle that includes a sexual and an asexual phase of spore production. *Phytophthora* species are either homothallic (self-fertile), having the ability to undergo sexual reproduction with only one individual, or heterothallic, meaning the species requires two mating types for sexual reproduction (meiosis and recombination), although homothallic isolates have been found in species previously identified as heterothallic and some homothallic species can outcross (Goodwin 1997). Regardless of whether reproduction is homothallic or heterothallic, the reproductive outcome is a thick-walled, sexual spore called an oospore, which functions not only as a means to recombine genetic material, but also as a structure that can survive adverse environmental conditions.

Phytophthora reproduction also can occur asexually through the production of sporangia that can germinate directly or develop and release multiple, motile zoospores that are capable of swimming to a potential host and forming a cyst that germinates and then penetrates host tissue. Additionally, chlamydospores can be formed, which are asexual overwintering structures that are able to persist through mildly adverse environmental conditions better than sporangia, but not as well as oospores. These multiple (and elastic) reproductive strategies allow *Phytophthora* species to adapt readily to their environments. As a result of this genetic elasticity, plant pathogens like *Phytophthora* are notorious for their ability to quickly evolve fungicide resistance (Goodwin and others 1996) and overcome host plant resistance to exploit new opportunities (Fry 1982). For example, new races of potato late blight (caused by *P. infestans*) quickly destroyed single major resistance genes in potato (Fry 1982) and widespread resistance to metalaxyl has rendered the fungicide ineffective in commercial control of late blight (Goodwin and others 1996).

The multiple reproductive strategies of *Phytophthora* species may partially explain their success as important plant pathogens of agricultural and ecological systems and can account for significant losses due to root rots, crown rots, leaf blights, cankers, and stem dieback (Leonberger and others 2013; Erwin and Ribeiro 1996). Although many *Phytophthora* species have limited host ranges, similar to what is seen for the causal organisms of chestnut blight and Dutch elm disease, other *Phytophthora* species have a very broad host range that includes a diversity of host plants. These host plants often grow in proximity to each other in nurseries, greenhouses (Leonberger and others 2013), and later, in the landscape and forest ecosystem (Hansen and others 2012; Frankel 2008; Rizzo and others 2002; Zentmeyer 1983).

This diversity of hosts is mirrored in species diversity of the genus *Phytophthora*, which currently includes more than 100 described members capable of infecting more than 1000 plant hosts, causing significant economic and ecological losses around the world (Kroon and others 2012; Erwin and Ribeiro 1996). Historically, the identification of *Phytophthora* species was based on morphology. Waterhouse (1963) developed a key that divided the genus into six groups, based on host range, sporangium types, antheridium and oogonium morphology, chlamydospore production, observation of hyphal swellings, optimal growth temperature, and colony morphology. Many of these characters are not produced consistently and can be very variable in nature and in culture. It is not surprising, therefore, that *Phytophthora* isolates in culture are difficult to identify to species based solely upon morphology. This difficulty was acknowledged early on, and it was suggested that naturally occurring interspecific hybrids of *Phytophthora*, if they even existed, would be difficult to identify (Brasier 1991). However, during the last decade, recent advances in molecular techniques and their incorporation into molecular taxonomy have resulted in a dramatic expansion of the genus *Phytophthora*, with the number of recognized species nearly doubled, and new species and hybrid species identified regularly (Cooke and others 2000; Kroon and others 2004; Martin and Tooley 2003; Man in't Veld and others 2012). This explosive increase in *Phytophthora* species identification is due primarily to the application of molecular tools to taxonomy, which has provided greater resolution and an improved understanding of the species concept in this genus (Hansen and others 2012; Kroon 2010). In addition to identifying new species, these molecular techniques have also proven the putative hybrid nature of atypical strains (Brasier and others 1999; Brasier and others 2004).

Although most fungi occupy relatively limited geographic ranges (Ellison and others 2011; Giraud and others 2010), they may have the potential to occupy much larger ranges if dispersal barriers are overcome (Springer and Chaturvedi 2010), as they are via nursery trade. The dispersal of pathogenic fungi by humans has been linked to the migration of humans with their plants and animals, and to the global trade in food and other products (Stuckenbrock and others 2008; Brasier 2008). Thus the potential for the rapid evolution and spread of pathogenic species appears to be substantial, posing serious threats to wild plant and animal species, to food security, and to ecosystem health (Fisher and others 2012). However, the expansion of geographic ranges may be only the beginning of large-scale plant disease epidemics (epiphytotics). The recent identification of new *Phytophthora* species in the nursery industry (De Cock and Levesque 2004) has been followed by the identification of hybrids (Bonants and others 2000) and further dissemination of these new *Phytophthora* hybrids via the nursery trade (Man in't Veld and others 2007). For example, the hybrid *P. nicotianae X cactorum* was first identified in the Netherlands, but now has been found throughout Europe, Hungary, Peru, Taiwan (Érsek and Man in't Veld 2013) and in the US (Leonberger and others 2013). The discovery of widespread *Phytophthora* hybrids has brought about a recognition that the introduction of exotic species is not the only contributor to epiphytotics.

Rise of the Hybrid Phytophthoras

A hybrid refers to an offspring that is the result of breeding between two different species: A cross between a horse and a donkey results in a hybrid called a mule; a cross between European honeybees and African bees resulted in the 'killer bees.' In plants, hybrids are regularly developed to improve specific characteristics in the resulting seeds, and to protect against deleterious characteristics due to the likelihood of different alleles (heterozygosity) at a given gene or locus. Between species, reproductive barriers exist to prevent hybridization from occurring regularly, as it would most likely result in the wasting of gametes by producing unadapted offspring. These barriers are usually stronger when the species occur in the same geographic area and regularly encounter another (sympatry) than when the species have evolved in isolation (allopatry). Historically, and in the context of animal evolutionary biology, interspecific hybridization, at least in nature, is a rare event, but see work in plants (Riesenberg and others 2003) and fungi (Frey and others 1999; Newcombe and others 2000) for exceptions. In *Phytophthora*, an organism known for its genetic elasticity, population studies in greenhouses found only two species of Phytophthora contributing to the disease outbreaks in the affected greenhouses, and that asexual reproduction of the pathogens drove the epidemics (Lamour and others 2003), highlighting the rarity of hybridization.

Despite its relative rarity, interspecific hybridization has been proposed as a key mechanism in the evolution of invasive species (Ellstrand and Schierenbeck 2000; Schierenbeck and Ellstrand 2009). The rapid evolutionary change that results from hybridization has an obvious and important role in the process in biological invasions (Prentis and others 2008). Schierenbeck and Ellstrand (2009) reported 35 examples in 16 plant families in which invasiveness followed interspecific hybridization. Multiple introductions of a non-native species, particularly from a wide geographic range, may also create opportunities for rapid evolutionary change through interspecific hybridization (Schierenbeck and Ellstrand 2009). There is no reason to think this cannot happen with *Phytophthora*, and considerable evidence suggests, at least in the case of alder decline, that it already has (Brasier and others 2004). A hybrid ultimately described as *P. alni*, first detected on dying alders and associated with 10-15% mortality of alders in Great Britain, has spread throughout Europe. Originally thought to be a hybrid of *P. cambivora* and a *P. fragariae*-like species (Brasier and others 1999), the original hybridization event itself is open to conjecture; but agreement exists that the event was recent, and that *P. alni* arrived in Britain on infected nursery stock. It is unknown how this hybrid arrived in Alaska, and it continues to be a threat to alder species, and other *Phytophthora*-susceptible trees (Brasier and others 2004).

In 1998, an unknown *Phytophthora* isolate was recovered from diseased ornamentals in the genera *Spathiphyllum* and *Primula* grown hydroponically in the Netherlands (Man in 't Veld and others 1998). These isolates were described as natural hybrids between *P. nicotianae* and *P. cactorum* (Man in 't Veld and others 1998). In 2000, additional atypical *Phytophthora* isolates were recovered on different hosts. DNA fingerprinting demonstrated that these isolates were also *P. nicotianae* x *P. cactorum* hybrids and that they likely had emerged through different hybridization events (Bonants and others 2000). Continuous hydroponic systems with multiple crops provide an ideal environment for *Phytophthora* species to co-exist on a variety of different hosts where they may eventually be able to hybridize (Bonants and others 2000). Other *P. nicotianae* x *P. cactorum* hybrids have been found on loquat (*Eriobotrya japonica*) in Taiwan and Peru, but with genetically distinct isolates (Chern and others 1998; Hurtado Gonzales and others 2009).

Many currently identified hybrids have resulted from interspecific hybridization within clades of closely related species (Kroon and others 2010). *P. nicotianae* and *P. cactorum* are closely related species (Cooke and others 2000), as are *P. hedraiandra* and *P. cactorum* (Kroon and others 2010). This suggests that the evolution of some *Phytophthora* hybrids may have been possible because the two parent species have not diverged to a point where they are incompatible (Man in 't Veld and others 1998). To date, most *Phytophthora* hybridizations that have been identified appeared to have occurred between species that evolved allopatrically in different geographical locations, involving an exotic and native species, or two exotic species that occupied the same niche (Érsek and Man in't Veld 2013).

Hybrid *Phytophthoras* and Transgressive Segregation

Within any group of organisms, and especially within the genus *Phytophthora*, extraordinary variation exists, and multiple mechanisms are in place to ensure the organism's persistence. Within the field of ecology, two highly polarized viewpoints exist: Hybridization serves as "a potent evolutionary force that creates opportunities for adaptive evolution and speciation and provides increased genetic variation and new gene combinations that promote the development and acquisition of novel adaptations"; or hybridization contributes little in evolutionary terms (aside from allopolyploidy), serving as a primarily local phenomenon with only transient effects (Riesenberg and others 2003, p. 1.

Within the laboratory, synthetic hybridization experiments have been undertaken to examine the likelihood and outcome of such hybrids in plants (Riesenberg and others 2003), and in *Phytophthora* species (Érsek and others 1995; Goodwin and Fry 1994). In some instances, the resulting hybrid has had a modification of host range, with a loss of pathogenicity as compared to the parental isolates, or an additive effect where the hybrid had the ability to infect hosts of both parents, which did not have an overlapping host range (Érsek and others 1995; Goodwin and Fry 1994). Goodwin and Fry (1994) crossed the closely related *P. infestans* and *P. mirabilis*; both have limited host ranges. These two sympatric *Phytophthora* species have similar morphology, growth characteristics in culture and in planta, and high degree of genome homology (Kroon 2010). Most of the hybrids lost the ability to infect the hosts that were infected by the parents. However, a recent cross between an isolate of *P. infestans* virulent on potato and tomato (Solanaceae) and a *P. mirabilis* isolate virulent on the ornamental plant four o'clock flower (*Mirabilis jalapa*) (Nyctaginaceae) produced F1 and F2 progeny that were pathogenic on tomato, including one F2 isolate that was capable of infecting all parental hosts (Kroon 2010). Érsek and others (1995) created interspecific hybrids between *P. capsici* and *P. nicotianae* by zoospore fusion and found hybrids could infect either all previous parental host species (2/3), or none (1/3).

Although many hybrids are not viable, large phenotypic changes are possible. These changes can drive range expansion, host jumping in the case of pathogens, and increased virulence (Mallett 2007). Studies of quantitative traits in segregating hybrid populations often report phenotypes that are extreme or novel relative to those of either parental line (Goodwin and Fry 1994; Riesenberg and others 2003; Stuckenbrock and others 2012). These extreme or novel phenotypes are described as transgressive segregants (Riesenberg and others 1999) and may exhibit traits (dispersal, resource acquisition, stress tolerance) that allow them to overcome biotic and abiotic obstacles that constrained the parental lines. Thus, transgressive segregation can contribute to invasiveness. This was demonstrated with hybrid Phytophthora isolates from alders in the UK (Brasier and Kirk 2001). The parents of the hybrids are believed to be *P. cambivora* and *P. fragariae*, neither of which is a strong pathogen of alder. However, the hybrids are highly pathogenic to alder so have an altered host range compared to either parent.

Transgressive segregation is an important process for generating novel traits that are heritable in both agricultural and native environments. Phytophthora species provide almost a model system for studying these mechanisms, due to the ability of the genus to tolerate different numbers and combinations of chromosomes, with triploids, tetraploids and many aneuploid types with odd numbers of chromosomes known (Goodwin 1997). The genomic plasticity of Phytophthora provides the genetic playground necessary to avoid potentially deleterious mutations. This plasticity also shelters the genetic material for transgressive segregation that can result in stable, hybrid isolates with traits that are very different from either parent (Kroon 2010; Érsek and others 1995).

Transgressive segregation is one means of explaining the expansion of host range observed with the *P. cactorum x hedraiandra* isolates in the US. Originally reported on *Rhododendron*, and later *Viburnum*, in Europe (Man in't Veld and others 2012) and the US (Leonberger and others 2013), an isolate of this hybrid was found on bleeding heart (*Dicentra)* in a nursery, a host that had not been reported for either parental species. Koch's postulates were performed to confirm that the hybrid could infect the original *Dicentra* cultivar 'Luxuriant.' Since the parental species were not able to infect the *Dicentra* host, an expansion of host range through hybridization was proposed (Leonberger 2010). Subsequent studies found that the hybrid isolate could infect additional species of native bleeding hearts, including wild bleeding heart (*D. eximia*), squirrel-corn (*D. canadensis*), and Dutchman's breeches (*D. culcullaria*) (Beckerman and Gerberich, unpublished). It has been hypothesized that *Phytophthora* hybrids often arise as offspring of two exotic species or of an exotic and resident species (Man in't Veld and others 2007; Érsek and Nagy 2008). In this instance, an exotic and native *Phytophthora* were the putative parental species, and yet the offspring exhibited broader host ranges than either parent (transgressive segregation), resulting in new host range specialization and increased pathogenicity and virulence (Brasier 1995).

The Rhododendron as a Hybrid Zone

A hybrid zone is a location where species or interspecific lines comingle, mate, and produce hybrid offspring (Barton and Hewitt 1989). Hybrid zones that arise from the mingling of species or intraspecific lines that were previously isolated geographically may play an important role in invasions. Hybrid zones might also arise as a consequence of habitat disturbance and/or environmental change, and hybrids can certainly be found in disturbed habitats (Ellstrand and Schierenbeck 2000; Harrison 1993). However, we propose that nurseries, greenhouses, and landscaping can serve as hybrid zones for *Phytophthora* by placing hosts for native and introduced species in close proximity. The spread of *Phytophthora* species between production facilities via plant movement has been documented (Lamour and others 2003) and is understood to be a means of invasion (Rizzo and others 2002 ; Brasier and others 2001) for the pathogen. More specifically, we suggest that widely planted species like *Rhododendron* may themselves serve as hybrid zones. *Rhododendron* species (including azaleas, which belong to the genus *Rhododendron*), hybrids, and cultivars are popular and widely planted ornamentals. Within the nursery industry, rhododendron is recognized as being regularly infected by a number of *Phytophthora* species known to cause root rot (*P. cactorum, P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. drechsleri, P. gonapodyides,* and *P. megasperma*), shoot blight (*P. cactorum, P. citricola, P. citrophthora, P. hedriandra, P. cactorum x hedriandra, P. nicotianae, P. cactorum X nicotianae, P. ramorum, P. syringae*), leaf spot (*P. syringae*) and damping off of seedlings (*P. cactorum, P. cinnamomi,* and *P. cryptogea*) (Hoitink and Schmitthenner 1974; Benson and Jones 1979; Erwin & Ribeiro 1996; Werres 2000; Farr and others 1996). In several *Phytophthora* surveys, rhododendron is commonly found as the host genus supporting the greatest number of isolates and diversity of *Phytophthora* species (Leonberger and others 2013; Yakabe and others 2009, Schwingle and Blanchette 2008).

Riesenberg and others (2003) found that hybridization can play an important role in adaptive evolution, but only if fit hybrid genotypes can escape from “the mass of unfit recombinants” in a hybrid population. A host plant like Rhododendron can serve as a hybrid zone because it supports the growth of multiple *Phytophthora* species without restriction. This lack of selection is necessary for hybridization to occur, for the establishment of hybrid offspring, and subsequent genotype fixations that are required for new, hybrid populations to evolve. Thus, *Rhododendron* species (and others like *Viburnum* and *Pieris*) serve as a hybrid zone, bridge, and Trojan horse of *Phytophthora* to the landscape, and are in and of themselves, a critical control point in need of better management (Parke and Grunwald 2012).

One aspect that drives the success (and the concerns about) fungal hybridization is that many fungal pathogens undergo recurrent cycles of asexual reproduction after intermittent sexual cycles. Thus, the establishment of hybrid species is facilitated by the multiple cycles of asexual reproduction in a saprophytic stage that provide inoculum build-up prior to selection for pathogenicity. The mixture of sexual and asexual reproduction present in many plant pathogens, and especially *Phytophthora* species, may facilitate the creation of new genetic combinations and the rapid amplification prior to and after successful infection of a new host.

Management to Minimize *Phytophthora*

There are several practices that can be taken to minimize the introduction, establishment, spread and potential hybridization of *Phytophthora* in the nursery. These practices are conceptually simple, but may be difficult to implement, First, nursery owners should know the source of their material and whether common sanitation practices are followed. Nursery owners should recognize that some plants are more susceptible to *Phytophthora* and require greater care. In the forest nursery, these highly susceptible plants include *Fagus, Juglans, Malus, Quercus,* and many conifers, including but not limited to *Abies, Chamaecyparis, Picea, Pinus, Thuja,* and *Tsuga*. Many native understory shrubs are even more susceptible than trees, especially *Ilex, Kalmia, Pieris, Rhododendron*, and *Viburnum*. This is especially important with *Rhododendron* and *Kalmia* species, which are extremely susceptible to and can tolerate infection from multiple *Phytophthora* species. Second, new plants should be placed under temporary quarantine in a separate area from regular stock. It is critical to NOT treat them with fungicides effective against *Phytophthora* during the quarantine stage; fungicides suppress and delay symptom development, but will not ‘cure’ infected plants. Using fungicides at this stage will mask symptoms and allow infected plants to remain in the nursery instead of being culled. Instead, nursery owners should observe the quarantined plants for several weeks, and dispose of any plants showing symptoms of disease by burning (do not compost). Third, containers should never be placed directly on soil where *Phytophthora* may be present; *Phytophthora* can readily move from the soil and any debris onto plants. Ideally, nursery ground should be sloped or well draining with 3 to 4 in (7.5 to 10 cm) of coarse gravel or rock between the soil and containers. This step raises containers from the ground, preventing infection from any soil-borne *Phytophthora*, and ensures good drainage at the site, to prevent waterlogged soil and standing water. Drainage tile, or a five-percent slope from gravel, can provide sufficient drainage to further prevent water from puddling. Ideally, slopes and tile should drain to irrigation channels that release water to a central holding pond. Water in this pond should be treated as containing *Phytophthora*, and subject to water treatment to minimize the amount of *Phytophthora*. Effective methods of treatment include chlorination, copper/silver ionization, slow sand filtration and ultraviolet radiation.

Finally, weeds, sick plants, or debris that can harbor pathogens or pests in the nursery and planting beds should be removed. Tools should be cleaned and sterilized regularly, as well as benches. If pots are being recycled, be sure to sterilize them between crops. Pots need to be well scrubbed and disinfected. Ideally, disinfection can be done with aerated steam via an autoclave; alternatively, use of a commercial disinfectant, which contains both antimicrobials and detergents (the detergent breaks down the cell wall to allow the disinfectant to better penetrate) for effective sterilization, is recommended. Any step that minimizes the likelihood of *Phytophthora* establishment minimizes the possibility of hybridization between species.

The High Price of Free Trade

The introduction of exotic invasives, including plant pathogens, is an under-recognized ecological problem caused by the globalization of commerce. Plant imports in the US increased 33% per decade over the past 43 years, and the importation of live plants is the most common pathway for the introduction of non-native plant pathogens, which costs US taxpayers billions of dollars annually (Liebhold and others 2012; Aukema and others 2011; Pimentel and others 2005). However, once here, the problem can extend beyond the scope of the primary introduction due to hybridization events, creating new pathogen diversity, and new hosts. Hybridization has already been recognized as a mechanism for invasiveness in plants (Schierenbeck and Ellstrand 2009), and in *Phytophthora* species (Brasier and others 2001). Regulatory programs on a national and international scale need resources to monitor and restrict "the predictable pathways by which pathogens move" (Hansen 2008 p. 40) and recognize those genera, like *Rhododendron*, *Pieris* and *Viburnum*, which serve as either reservoirs or even Trojan horses that promote pathogen spread and hybridization, and monitor these hosts more closely to minimize risk.

References

Aukema J, Leung B, Kovacs K, and others 2011. Economic impacts of non-native forest insects in the United States. *PLoS ONE* 6: e24587.

Barton NH, Hewitt, GM. 1989. Adaptation, speciation and hybrid zones. Nature 341: 497-502

Benson DM, Jones RK. 1979. Etiology of Rhododendron dieback caused by four species of *Phytophthora*. Plant Disease 64:687-691.

Bonants PJM, Hagenaar-de Weerdt M, Man in 't Veld WA, Baayen RP. 2000. Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. Phytopath. 90:867-874.

Brasier CM. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57: 792–808.

Brasier CM, Kirk SA, Delcan, J., Cooke, D. E. L., Jung, T., and Man in 't Veld, W. A. 2004. *Phytophthora alni sp. nov*. and its variants: Designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycol. Res. 108:1172-1184.

Brasier CM, Kirk SA. 2001. Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. Plant Pathology 50: 218-229.

Brasier CM, Cooke, DEL, Duncan JM. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proc. Natl. Acad. Sci. USA 96:5878-5883.

Brasier CM. 1995. Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Can. J. Bot. 73:1213–1221.

Brasier CM. 1991. Current questions in *Phytophthora* systematics: The role of the population approach. In *Phytophthora* (J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, Eds.), pp. 104–128. Cambridge Univ. Press, Cambridge.

Chern LL, Ann PJ, Young HR. 1998. Root and foot rot of loquat in Taiwan caused by *Phytophthora*. Plant Dis. 82: 651–656.

Cooke DEL, Drenth A, Duncan JM, Wagels G, Brasier CM. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genet. Biol. 30:17–32,

De Cock AWAM, Lévesque A. 2004. New species of Pythium And *Phytophthora*. Studies in Mycology 50, 481–7.

Ellison CE, Hall C, Kowbel D, and others 2011. Population genomics and local adaptation in wild isolates of a model microbial eukaryote. Proc. Natl Acad. Sci. USA 108: 2831–2836.

Ellstrand NC, Schierenbeck K. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. USA 97:7043-7050.

Érsek T, Man in't Veld WA. 2013. *Phytophthora* species hybrids: A novel threat to crops and natural ecostems. In *Phytophthora*, A Global Perspective.Lamour, K. Editor.

Érsek T, Nagy ZÁ. 2008. Species hybrids in the genus *Phytophthora* with emphasis on the alder pathogen *Phytophthora alni*: a review. Eur. J.of Plant Pathol., 122, 31–39.

Érsek T, English JT, Schoelz JE. 1995. Creation of species hybrids of *Phytophthora* with modified host range by zoospore fusion. Phytopath. 85:1343-1347.

Erwin DC, Ribeiro OK. 1996. *Phytophthora* Diseases Worldwide. St. Paul, MN: APS Press. 562 pp.

Farr DF, Rossman AY. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved October 31 2013, from http://nt.ars-grin.gov/fungaldatabases/ and 1996 print edition.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McGraw SL, Gurr SJ. 2012. Emerging fungal threats to animal, plant, and ecosystem health. Nature 484:186-194.

Frankel SJ. 2008. Sudden oak death and *Phytophthora ramorum* in the USA: a management challenge. Australasian Plant Pathology 37:19–25.

Frey P, Gatineau M, Martin F, Pinon J. 1999. Molecular studies of the poplar rust *Melampsora medusae-populina*, an interspecific hybrid between *M. larici-populina* and *M. medusae*. Page 34 in Proceedings of the International Poplar Symposium III; September 13–17 1999; Orleans (France).

Fry WE. 1982. Principles of Plant Disease Management. Academic Press, NY. 378 p.

Giraud T, Gladieux P, Gavrilets S. 2010. Linking the emergence of fungal plant diseases with ecological speciation. Trends Ecol. Evol. 25: 387–395.

Goodwin SB. 1997. The population genetics of *Phytophthora*. Phytopath. 87: 462–473.

Goodwin SB, Fry WE. 1994. Genetic analyses of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. Exp. Mycol. 18: 20-32.

Goodwin SB, Sujkowski LS, Fry WE 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. Phytopath. 86:793-800.

Hansen EM. 2008. Alien forest pathogens: *Phytophthora* species are changing world forests. Boreal Environment Research 13:33-41.

Hansen EM. Reeser, P.W., and Sutton, W. 2012. *Phytophthora* beyond agriculture. Annu. Rev. Phytopathol. 2012.50:359-378

Harrison RG. 1993. Hybrids and Hybrid zones: Historical Perspective. In: Hybrid Zones and the Evolutionary Process. Oxford University Press. Pp 3-12.

Hoitink HAJ, Schmitthenner AF. 1974. Relative prevalence and virulence of *Phytophtora* species involved in Rhododendron root rot. Phytopath. 64: 1371-1374.

Hurtado-Gonzales OP, Aragon-Caballero LM, Flores-Torres JG, Man in 't Veld WA, Lamour KH. 2009. Molecular comparison of natural hybrids of *Phytophthora nicotianae* and *P. cactorum* infecting loquat trees in Peru and Taiwan. Mycologia

Kroon LPNM, Brouwer H, de Cock, AWAM, Govers F. 2012. The genus *Phytophthora* anno 2012. Phytopat. 102:348-364.

Kroon, LPNM. 2010. The genus *Phytophthora*; phylogeny, speciation and host specificity. Thesis, Wageningen University, Wageningen, NL . ISBN 978-90-8585-668-9.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM, Flier WG. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear sequences. Fung. Genet. Biol. 41:766-82.

Lamour KH, Daughtrey ML, Benson DM, Hwang J, Hausbeck MK 2003. Etiology of *Phytophthora drechsleri* and *P. nicotianae* (=*P. parasitica*) diseases affecting floriculture crops. Plant Dis. 87: 854–858.

Leonberger AC, Speers Ruhl G, Creswell T, Beckerman JL. 2013. A Survey of *Phytophthora* spp. in Midwest Nurseries, Greenhouses, and Landscapes. Plant Dis. 97:63–40.

Leonberger AJ. 2010. Distribution and host specificity of Phytophthora species found in Indiana nurseries, greenhouses, and landscape plantings. M.S. Thesis, Purdue University, West Lafayette, IN. http://docs.lib.purdue.edu/dissertations/AAI1490674/

Liebhold AM, Brockerhoff EG, Garrett LJ, Parke JL, Britten KO. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. Front Ecol. Environ. 10: 135–143

Mallet J. 2007. Hybrid speciation. Nature 446:279–283.

Man in 't Veld WA, de Cock AWAM, Summerbell RC. 2007. Natural hybrids of resident and introduced *Phytophthora* species proliferating on new hosts. Eur. J. Plant Pathol. 117:25–33.

Man in 't Veld WA, Rosenthal CHM, Hong C. 2012. *Phytophthora x serendipita* sp. Nov and *P. x pelgrandis*, two detructive pathogens generared by natural hybridization. Mycologia 104: 1390-6.

Man in 't Veld WA, Veenbaas-Rijk WJ, Ilieva E, de Cock AWAM, Bonants PJM, Pieters R. 1998. Natural hybrids of *Phytophthora nicotianae* and *P. cactorum* demonstrated by isozyme analysis and random amplified polymorphic DNA. Phytopath. 88:922–929..

Martin FN, Tooley PW 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. Mycologia 95: 269-284.

Newcombe G, Stirling B, McDonald S, Bradshaw JR. 2000. *Melampsora* x *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. Myco. Res. 104: 261–274.

Parke JL, Grünwald NJ. 2012. A systems approach for management of pests and pathogens of nursery crops. Plant Dis. 96:1236-1244.

Pimentel D, Zuniga R, Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52: 273–88.

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008) Adaptive evolution in invasive species. Trends in Plant Science 13: 288–294.

Riesenberg LH, Raymond O, Rosenthal DM, Lai Z., Livingstone K., Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, and Lexer C. 2003. Major ecological transitions in annual sunflowers facilitated by hybridization. Science 301:1211-1216.

Rieseberg LH, Archer MA, Wayne RK. 1999. Transgressive segregation, adaptation, and speciation. Heredity 83:363-372.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis. 86:205-214.

Schierenbeck KA, Ellstrand NC. 2009. Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions 11: 1093-1105

Schwingle BW, Blanchette RA. 2008. Host range investigations of new, undescribed,and common *Phytophthora* spp. isolated from ornamental nurseries in Minnesota. Plant Dis. 92:642-647.

Springer DJ, Chaturvedi V. 2010. Projecting global occurrence of *Cryptococcus gattii*. Emerg. Infect. Dis. 16: 14–20.

Stukenbrock EH, McDonald BA. 2008. The origins of plant pathogens in agroecosystems. Annu. Rev. Phytopathol. 46: 75–100.

Stuckenbrock EH, Christiansen FB, Hansen TT, Dutheil JY, Shierup MH. 2012. Fusion of two divergent fungal individuals led the recent emergence of a unique widespread pathogen species. Proc. Natl. Acad. Sci. (USA) 109:10954-10959

Waterhouse GM. 1963. Key to the species of *Phytophthora* de Bary. *Mycology Paper* No. 92, 22 pp. CMI Kew, UK.

Werres S, Marwitz R, Man in 't Veld WA, De Cock AWAM, Bonants PJM, DeWeerdt M, Themann K, Ilieva E, and Baayen RP. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycol. Res. 105:1155-1165.

Yakabe, LE, Blomquist CL, Thomas SL, MacDonald JD. 2009. Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. Plant Disease 93:883-890.

Zentmyer GA. 1983. The world of *Phytophthora*. In: *Phytophthora*, its biology, taxonomy, ecology and pathology (Ed. by Erwin, D.C.; Bartnicki-Garcia, S.; Tsao, P.H.), pp. 1-8. American Phytopathological Society, St. Paul, USA.