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Biology, epidemiology, and control of *Heterobasidion* species worldwide

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Running title: *Heterobasidion* root and butt rots

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Abstract

Heterobasidion annosum sensu lato is a species complex comprising five species widely distributed in coniferous forests of the northern hemisphere, each characterized by distinct host preference. Over 1700 papers have been published on these fungi in the last decades, making them perhaps the most widely studied forest fungi. *Heterobasidion* species are at different levels on the saprotroph-necrotroph gradient, and the same individual can switch from one mode to the other. This offers a unique opportunity to study how genomic structure, gene expression, and genetic trade-offs may all interact with environmental factors to determine the life mode of the organism. The ability to infect

stumps as saprotrophs and to spread to neighboring trees as pathogens has resulted in significant damages to timber production in managed forest. This review focuses on the current knowledge on the biology, ecology, evolution, and management of these species based both on classical and modern studies.

1. Introduction

The genus *Heterobasidion* (Basidiomycota, Russulales, Bondarzewiaceae) included, until recently, five taxonomic species: *H. annosum* (Fr.) Bref., *H. araucariae* P.K. Buchanan, *H. insulare* (Murrill) Ryvarden, *H. pahangense* Corner and *H. rutilantiforme* (Murrill) Stalpers. The number of species has increased since both *H. annosum* and *H. insulare* have been reported to be species complexes (15–16, 84, 90).

While most *Heterobasidion* species are strict saprotrophs or display uncertain pathogenicity (15, 90), the *H. annosum* species complex, hereafter referred as *H. annosum* sensu lato (s.l.), comprises necrotrophic pathogens regarded as the most destructive disease agents of conifers.

In the last 40 years, *H. annosum* s.l. has been the object of over 1700 scientific papers, making it one of the most intensively studied forest fungi. The complete genome sequence of the fungus is now available, making *H. annosum* s.l. the first sequenced plant pathogenic homobasidiomycete (94). Furthermore, it is one of the few examples of forest pathogen that can and has been controlled in managed forests.

The biology, epidemiology, impact and control of *H. annosum* s.l. were the object of a book (144), while more recent reviews have an emphasis either on biochemistry and molecular aspects of pathogen virulence (3) or on epidemiology and disease management (46). This review attempts to present those highlights that make *Heterobasidion* an ideal model system to study basic and applied aspects of the biology, ecology and evolution of a forest pathogen. Not only does the complex include species that have diverged at different times, but the model is enriched by a combination of allopatric and sympatric distribution of several species, allowing to study the reinforcement of speciation processes and horizontal gene transfer. The complex includes species that not only are at different levels on the saprotroph-necrotroph gradient, but the same individual can often switch from one mode to the other. This offers a unique opportunity to study how genomic structure, gene expression, and genetic trade-offs may all interact with environmental factors to determine the life mode of the organism at any given time. Milestone traditional genetic studies have been published

(11–12, 50–51, 59–60, 111–112) elucidating patterns and heritability of genes involved in vegetative compatibility, mating, dikaryosis, and intersterility between species, providing a solid foundation for an in depth analysis using genomic and transcriptomic approaches. Quantitative analyses of traits regulating virulence and intersterility provide a roadmap for the search of loci affecting these important traits, and early molecular work is already available describing genes interacting between host and pathogen during the early stages of infection. Finally, the significant amount of research aimed at understanding the impact that this group of organisms has on production forestry, and, since 2004, the understanding that two sister allopatric species are now sympatric due to a human-linked intercontinental movement of one of the two, provide the scientific community with the opportunity to understand in depth the ecological similarities and differences among species within the complex.

2. Taxonomy and distribution of the complex *Heterobasidion annosum*: biological, phylogenetic or traditional species?

Heterobasidion annosum s.l. is widely distributed in coniferous forests of the northern hemisphere, especially in Europe, North America, Russia, China, and Japan (17–18, 68, 72, 100, 146). *Heterobasidion annosum* has long been regarded as a single species, until mating experiments among different individuals revealed the occurrence of intersterile groups (ISGs) (7, 10, 67). Currently, three ISGs are recognized in Eurasia and two in North America; all are now formally described as species. Eurasian groups were described as *H. annosum* sensu stricto (s.s.), *H. abietinum* Niemelä & Korhonen and *H. parviporum* Niemelä & Korhonen (90), while North American groups were named *H. irregulare* (Underw.) Garbel. & Otrosina and *H. occidentale* Otrosina & Garbel. (100). Although defined on the basis of partial reproductive isolation and morphology, further supported by phylogenetic analyses performed with a range of markers (19, 52, 77, 98), these species are also characterized by a distinct host preference. *Heterobasidion annosum* s.s. mostly attacks pines (*Pinus* spp.), especially Scots pine (*Pinus sylvestris* L.), but can be associated with several other conifers and even some broadleaved tree species. *Heterobasidion parviporum* is strictly associated with Norway spruce [*Picea abies* (L.) Karst.], while *H. abietinum* is commonly associated with silver fir (*Abies alba* Mill.) and other species of the genus *Abies*. In North America, *H. irregulare* generally attacks pines, junipers (*Juniperus* spp.) and incense cedar [*Calocedrus decurrens* (Torr.) Florin], while *H. occidentale* shows a broader host range and can be found on the genera *Abies*, *Picea*, *Tsuga*, *Pseudotsuga* and *Sequoidadendron* (Table 1).

The distribution of *H. annosum* s.l. species reflects that of their main host species (Figure 1). *Heterobasidion annosum* s.s. is found all over Europe, except in the very northern regions and its distribution area extends east to the Altai region in southern Siberia (3, 17, 68). *Heterobasidion parviporum* occurs from the most northern parts of Europe to the southern Alps, and from western Europe to southern Siberia, China, and Japan (17, 18, 68–69, 140). *Heterobasidion abietinum* is found in central and southern Europe, and in the Mediterranean Basin (24, 73, 68, 81, 123). *Heterobasidion occidentale* is distributed only in western North America, from Alaska to southern Mexico (26 Filip and Morrison, 1998; 29 Garbelotto and Chapela, 2000), while *H. irregulare* is reported from both eastern and western North American forests (26, 100). The fungus is less common but can be found in the central part of North America (77). It can be found along with *H. occidentale* in the western United States (146). *Heterobasidion irregulare* was introduced into central Italy during World War II (47), and has since become invasive by spreading in Italian stone pine (*Pinus pinea* L.) stands (45). Therefore, the current distribution area of *H. irregulare* also includes an area of about 100 km along the Tyrrhenian coast west of Rome (20, 45).

The evolutionary history and biogeography of species within *H. annosum* s.l. were recently elucidated (19, 77). Speciation within the species complex began well after the radiation of host genera, about 60 million years ago (Ma). The pine-associated *H. annosum* s.s. and *H. irregulare* are sister taxa and appear to share a common origin in a Eurasian progenitor of all species within the complex. The last emerging species was *H. abietinum*, which arose 14–31 Ma. Although *H. annosum* s.l. species are characterized by partial interfertility in laboratory experiments and by overlapping ranges, interspecific hybrids have been reported rarely and only between the sympatric North American *H. irregulare* and *H. occidentale* (34, 80). Notwithstanding the difficulty of identifying hybrids or genotypes characterized by admixed genome, the lack of significant divergence in *Internal Transcribed Spacer* (*ITS*) among species (19, 52) and the clear evidence of horizontal gene transfer (77) indicate the species have had the opportunity of hybridizing and exchanging genes. In western North America, this opportunity has recently been fostered by the presence of pine or juniper stumps, colonized by both species (97, 34), but may have been facilitated in the past by the presence of larch (*Larix* spp.), a host that may be infected by both fungal species (80). Recent work on *Heterobasidion* mycoviruses has indicated that identical viral strains may be present in different species, further reinforcing the concept that interspecific exchanges of genetic material and of cytoplasmic factors do occasionally occur (141).

3. Biology, epidemiology and life cycle

3.1. Biology and life cycle

Studies conducted over 60 years ago elucidated the general mechanisms of infection by *Heterobasidion annosum* s.l. and identified it as having a mixed-infection biology (116–118). Primary infection is effected by airborne propagules (mostly basidiospores) on freshly exposed wood surfaces such as stump tops or stem and root wounds. Once established through primary infections, the fungus may spread and infect uninjured trees by vegetative growth of the mycelium through root contacts or grafts (secondary infection). Primary mycelium is mostly haploid and generated by the germination of single basidiospores. Secondary mycelium is actually a mosaic of haploid and heterokaryotic mycelia: the heterokaryotic portion of the thallus is generated by the mating of primary mycelia bearing different mating alleles, followed by frequent sectoring into haploid portions (50, 135). It appears that the incidence of haploid sectors may be directly correlated with the genetic distance between the two mating primary mycelia (111–112). Interestingly, a natural *H. irregulare* x *H. occidentale* hybrid found in California (34) was an obligate heterokaryon, without segregation of haploids either in the thallus or through uninucleate conidia (31), suggesting a diploid nature maybe necessary for its existence in nature.

Although the fungus produces both sexual spores and conidia, only the former seem to cause infections in nature (115). Such observation is supported by the absence of disjunct but genetically identical individuals (ramets) in forest stands. Identical genotypes generally occupy contiguous areas in forest stands, and consistently they have been better explained by secondary tree-to-tree infection rather than by conidial infection (30, 41, 137). Population genetics studies in diseased forests have also confirmed the importance of stump infection in the epidemiology of *H. annosum* s.l. by identifying the same fungal genotypes in stumps and in adjacent standing trees (9, 137).

Stumps remain susceptible to infection for a variable period of time depending on host, site, and season; however susceptibility rarely exceeds one month due to changes in the chemistry of nutrients available on stump tops and to competition by other microorganisms (115, 143).

Colonization proceeds downwards to the root system at a rate of up to 20 cm per month, depending on climate and host species (56, 115). In most species including pines, Norway spruce, and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] the sapwood of stumps is more readily colonized than the heartwood (115). Levels of wood moisture have been identified as major factors influencing patterns of stump colonization (113), but some evidence also points to a chemical nature of the heartwood of some species that may be a deterrent to fungal colonization (14).

Conversely, Shain (126) points to the inability of heartwood of Norway spruce trees (as opposed to sapwood) to respond to infection by accumulating phenolics and oleoresins as one of the major factors explaining the frequency of *Heterobasidion*-induced heartrot in this species. The probability of stump infection in general increases with increasing stump diameter (86), hence small stumps – such as those obtained in pre-commercial thinnings – are often regarded as inconsequential.

The probability of infection through wounds is related to the severity of wounding and to the host species. While there is no evidence that wounds on pines may represent significant infection courts – possibly because of wound-induced resinosis levels – Sitka spruce [*Picea sitchensis* (Bong.)

Carrière], western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], and occasionally Norway spruce have all been reported as susceptible to wound infection (115). Similarly, based on results of population studies, it was concluded that wounds rather than stumps provide the most common infection courts for *H. occidentale* in white fir [*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr.] and grand fir [*A. grandis* (Douglas ex D. Don) Lindl.] in western North America (30, 79). Wound infections may thus play a significant role in the life cycle *H. annosum* s.l. in unmanaged forests where stumps may be rare or absent.

Heterobasidion annosum s.l. is not able to freely grow in the soil. Hence direct root contact among trees or among stumps and trees is pivotal for secondary infection to occur. Interestingly, root grafts have been shown to be less effective in stump-to-tree transmission than simple root contacts, due to the fact that stump roots grafted to tree roots tend to remain alive, thus confining the fungus to the inner wood (99). Depending on soil properties, the fungus may either directly colonize the bark of live roots (in low pH soils) or grow ectotrophically under the outer bark scales (in high pH soils) (136). Ectotrophic growth may result in significantly higher fungal expansion rates, due to the lack of an active host defense response.

3.2. Patterns and rates of secondary spread

A large number of small genets can generally be found in young plantations or first rotation forests, a pattern that is suggestive of a recent primary establishment of the fungus through infections via basidiospores (137). In old forest sites and in second or later rotation stands, larger genets may develop as the result of the spread of mycelium through root contacts. The largest genets ever reported measured 50 and 57 m, caused by *H. parviporum* in Japan and *H. abietinum* in Spain, respectively (123, 140). In most cases, the largest diameter of individual *H. annosum* s.l. genets is less than 30 m, and involve only a few trees (30, 41, 103–105, 110, 131). The fact that genet expansion is limited for North American *Heterobasidion* species was shown in a long-term study in

California, where the complete arrest of canopy gap expansion was documented in numerous instances (121).

Based on the largest reported extension of genets, and assuming an average spread of the fungus in the order of 20-200 cm year^{-1} (35, 121, 128, 136), the age of individual mycelia would range between 14 and 140 years, a much younger age than those reported for *Phellinus weirii* (Murrill) Gilb. (i.e. 1340 years old) (23) and *Armillaria* spp. (more than 1500 years old) (129). The limited size of genets indicates that, although secondary spread may be common for *H. annosum* s.l., this pathogen relies mostly on primary infection, and vegetative expansion is not epidemiologically as relevant as it is for other root disease fungi. Persistence of identical genets in different rotations suggests that intergenerational secondary infection may play a role in increasing disease incidence at the stand level (102, 132). However, increase in disease incidence is more convincingly linked to primary infection by basidiospores produced by fungal individuals established in the previous rotation (136).

Thinnings in Norway spruce stands have been suggested to promote not only primary infection but also secondary tree-to-tree spread of the pathogen. An acceleration of secondary growth of the fungus and an increase in tree-to-tree transmission through root contacts were in fact documented in the roots of felled trees, where lack of an active host response resulted in a much greater colonization of the outer root layer (4, 104).

3.3. Range and temporal patterns of primary spread

Although spores of *H. annosum* s.l. have been reported to travel long distances, i.e. hundreds of km (63, 120), there is an increasing body of evidence pointing to a geographical limited range of effective spore dispersal, i.e. spore dispersal resulting in fungal establishment. Based on data from Kallio (120), Stenlid (133) fitted a model describing the dispersal gradient of *H. annosum* s.l. in which only 0.1% of spores may travel 100 m. Other studies performed in regions as diverse as Fennoscandia, the Alps, and Mediterranean forests indicate effective spore dispersal to be between

98 and 1255 m (43–44, 87). This documented limited spread of viable basidiospores implies that during thinning operations or cuttings, the presence of basidiospore-producing fruitbodies increases the risk of stump infection within but not between forests. Low levels of spore movement among forests may be relevant only when dealing with the colonization of areas where *H. annosum* s.l. may not be present yet (43).

Temporal patterns of stump infection have been found to vary in different regions of the world. In northern Europe, infections follow a bell-shaped curve, with a maximum probability of infection during summer and with no infection during winter (6). In the UK, stump infections are documented to occur during most of the year (85, 119). In the Alps and in central Europe, the airborne inoculum of *H. annosum* s.l. can be detected as early as February at most sites, but normally peaks between August and October (42, 138). Such a trend might be common in continental climates, characterized by hot dry weather in summer combined with snow and low temperatures in winter. Less is known on the aerobiology of *H. annosum* s.l. in areas with a Mediterranean climate. In a study performed in central Italy, Garbelotto et al. (33) detected high levels of spore deposition of *H. annosum* s.s. in winter, and significantly lower levels in summer. In California's Sierra Nevada, sporulation was reported to occur all year round (58). In the southeastern USA, high summer temperatures reduce sporulation (25, 122), and also result in high stump temperatures that prevent infection (122). There is good evidence that spore production is primarily affected by temperature and humidity (115), and the average minimum air temperature of a four-week period has been identified as a suitable predictor variable for modeling *H. annosum* s.l. spore deposition (42).

3.4. Factors affecting disease incidence

Disease incidence is reported to increase with stand age (139) at rates depending both on host species and silvicultural management techniques (140).

Site location, soil type, and site history have all been reported to affect disease incidence. While correlations between disease incidence and site elevation, slope, and aspect are not always concordant (39, 72, 83, 139), correlations between soil type and spread of the fungus are consistent. Damage caused by *H. annosum* s.l. is generally greater on fertile soils and on sandy soils with a low organic matter content (1, 72). Furthermore, high calcium content and pH greatly influence the occurrence of the disease, by reducing the activity of antagonistic fungi in the soil. Results from long term experiments in Sitka spruce stands in UK clearly indicate that the rate of infection is much lower on deep peat soils than on mineral soils (114). Damages caused by the pathogen are generally greater on former agricultural lands and on former pastures than on forest soils (72).

4. Genetics, genomics and host-pathogen interactions

As early as 1967, the first investigations on how trees react to infection by *H. annosum* s.l. were published (125), providing evidence that woody plant hosts identified infection by *Heterobasidion* not only when attacked in their cambial region, but also in the wood. A reaction zone was described ahead of the front of fungal colonization in the sapwood and was found to be rich in phenolic compounds capable of inhibiting growth of *Heterobasidion* (126). As of 2012, several studies have confirmed this response both in wood and the cambium and identified lignin and phenolics as some of the antimicrobial compounds that accumulate in infected wood, ahead of the fungus (27). Up-regulation of genes involved in the biosynthesis of lignin (148), phenolics (21), but also some classes of host chitinases (22, 54) and peroxidases (22) have instead been reported in infected cambial layers. On the pathogen side, genes encoding mitochondrial proteins, several cytochrome P450, a vacuolar ATP synthase, and genes involved in basic cellular processes, and lignin and cellulose degradation, appears to be expressed during early stages of the infection process (64, 147), suggesting an important role of these genes in facilitating the pathogenic activity of *Heterobasidion* spp.

The sequencing of the entire genome has facilitated our understanding of pathogenesis related mechanisms both from a host-pathogen interaction and from an evolutionary perspective. Gene annotation and its subsequent validation have highlighted the presence of three batteries of genes, only partially overlapping, involved in saprotrophic growth, parasitic infection, and reproduction (94). Up-regulation of enzymes capable of utilizing simple sugars and of degrading cellulose is pivotal for the saprotrophic growth of the fungus. The necrotrophic phase instead requires the up-regulation of different genes, including those responsible for pectinolytic enzymes, secondary metabolite production, and the ability to sustain oxidative stress. While saprotrophic and necrotrophic growth require clearly distinct yet overlapping groups of genes, a rather different set of genes is expressed during the reproductive phase. The three sets of genes are not concomitantly expressed, suggesting a fine-tuned trade-off among them (94). It is clear that the impact and relevance of *H. annosum* s.l. have to do with the presence of all three sets of genes, and in particular with the ability to switch from a saprotrophic to a necrotrophic life style upon the detection of certain cues, likely to be associated with the nature of the substrate that is being colonized.

The presence of host specialized taxa within the *H. annosum* s.l. complex, has allowed to perform interspecific crosses to determine segregation patterns in the progeny in order to investigate which genomic regions may be involved in host-specific virulence. The earlier studies of Lind et al. (75–76) have allowed for mapping at least three of these regions on two distinct chromosomes, and currently, the availability of the genome, allows for a description of actual genes involved in pathogenesis. Interestingly, a large proportion of these genes are described as “orphan” because they appear to have no homology to other fungal genes with known function; additionally, genomic regions putatively associated with virulence appear to be rich in transposable elements, suggesting that – as it has recently been suggested for *Phytophthora ramorum* Werres, De Cock & Man in 't Veld (65) – virulence may be modulated at the gene expression level by activation or deactivation of these transposable elements. Virulence has also been proven to be associated with the mitochondrial genome, or at least with certain nuclear-mitochondrial combinations (32, 95): the

Heterobasidion mitochondrial genome is one of the largest ever sequenced for a fungus (94) and its sequence may allow to understand the reason for this observed correlation.

The potential discovery of new families of genes involved in pathogenesis, the role of transposable elements in modulating the pathogenic process, and the role played by the mitochondrial genome in determining virulence of a given genotype are three research areas that can be regarded as novel in the study of fungi and plant pathogens in general. *Heterobasidion* spp. have also long been system organisms to elucidate broader evolutionary processes such as the development of intersterility among populations leading to speciation, somatic compatibility (recognition of self), and a mating system displaying a continuum of compatibility depending on the relative phylogeographic history of each taxon within the complex. Classical genetic studies have allowed for the estimation of the number of loci involved in these processes (11–12, 50–51), and their identification may be now within reach thanks to the availability of the genome, possibly leading to a true leap in our understanding of fungal evolution.

5. *Heterobasidion* as an emerging pathogen: economic and ecological impacts

5.1. Role in natural forest ecosystems

In natural ecosystems *H. annosum* s.l. plays a subordinate role than that played in managed forest stands (127). However, *H. annosum* s.l. affects species composition, stand density and structure, and the direction and rate of forest succession (28, 37). When tree mortality occurs, gaps develop in the forest canopy, resulting in changes of light, moisture, and temperature in the forest. Furthermore, given its saprotrophic ability, the fungus is expected to contribute significantly to nutrient recycling.

5.2. Impact in intensively managed forests and plantations

In intensively managed forests, characterized by monocultures, plantation forestry, and, perhaps, establishment on former agricultural land, *H. annosum* s.l. greatly affects site productivity (70).

Heterobasidion annosum s.l. is the most common wood decay agent of conifers and represents a major threat to timber production, especially where intensive forest management is practiced. Financial losses caused by the species complex in the European Union were estimated at 790 million Euros per year (145). This estimate includes losses due to decay and overall reduction in diameter growth of infected trees (5) due to allocation of photosynthates to defense responses (i.e. reaction zone formation) rather than to growth (91), but it does not include windthrow and reduction of resistance of stands to storm damages caused by the fungus, which may be locally significant. Despite a remarkable regional variation in the frequency of root and butt rots, the incidence and losses caused by *H. annosum* s.l. are generally high. In the UK, incidence of decay was as high as 68% in Sitka spruce (106), with a loss in value of 43% (107). Disease incidence of Norway spruce in Alpine forests can locally be as high as 71%, with derived financial losses estimated between 18% and 34% (39). Once disease incidence increases above the "natural" background levels, it is virtually impossible to lower its threshold at values comparable to the original natural ones, but management strategies (see below) may ensure disease frequency does not increase. As for other root diseases, a strong interaction exists between root and bole infection by *Heterobasidion* species, insect attacks, and vigor affected by atmospheric pollutants, hence the overall effects of an increased presence of *Heterobasidion* in a forest stand cannot simply be evaluated on the basis of infection frequency alone (2, 57, 130). Effects associated with increases in incidence by *Heterobasidion* can include mortality on hosts rarely infected in natural conditions. In western North America, for instance, pine stumps can be colonized both by *H. irregulare* and *H. occidentale* allowing for an increased establishment of *H. occidentale* resulting in higher infection and mortality of true firs and of valuable sequoia, a tree species otherwise rarely affected by this pathogen (101).

5.3. Emergence in novel ecosystems

Until recently, the only evidence for human induced dispersal of *Heterobasidion* spp. was limited to short range movement of the organism through the use of infected fence posts (62). However, in 2004, the North American species *H. irregulare* was found to be infecting and killing Italian stone pines in a single location in Italy (47). Additional surveys (20, 45) showed the exotic species to be established in every Italian stone pine stand on 100 km of coastline west of Rome, as well as on Italian stone and Aleppo pines (*Pinus halepensis* Mill.) in some urban parks of the Italian capital (124). Although its native congener *H. annosum* s.s. has been long reported to be present in the region, prior to 2007 there was no information on the frequency and the role played by the native species in Tyrrhenian coastal pine stands. Based on published literature, *H. annosum* s.s. was to be considered relatively rare and causing limited mortality, with few and rare exceptions (8). Surveys aimed at describing the distribution of *H. irregulare* in central Italy determined that indeed *H. annosum* s.s. was rare both within and outside the *H. irregulare* zone of infestation, and only one stand out of 17 surveyed was found to harbor a significant population of the native species (45). However, every stand surveyed in the invasion area – even small groups of trees – harbored significant populations of the invasive organism, and tree mortality was apparent in clusters of varying sizes associated with presence of the exotic organism. Largest mortality clusters, including tens of dead trees, were found in Castelporziano and Castelfusano, where a possible introduction may have occurred in 1944 when regiments of the 5th US Army camped in the forest, leaving wood latrines and empty wood crates behind (47). Notwithstanding the fact that mortality rates may be affected by complex ecological factors, the extent of mortality in Castelporziano and Castelfusano is in agreement with a longer establishment of the invasive species. Additionally, most coastal pine stands are strikingly ecologically similar.

The newly attained sympatry between these two sister taxa provides the scientific community with an exciting opportunity to study the dynamics of a biological invasion in the presence of a closely related native species. Although all evidence points to a long (about 30 Ma) allopatric isolation between the two (19, 77, 98), *H. irregulare* and *H. annosum* s.s. have both been intensively studied, and their biology and epidemiology appear to be extremely similar. It is also known that mating systems have remained almost fully compatible in these two species evolved in allopatry (72, 134). However and unexpectedly, the two species have displayed a remarkable different adaptation to coastal habitats in central Italy where they are now sympatric. Inoculation experiments have indicated that both species are equally virulent on several European and North American pine species (33), hence lack of co-evolution is not a likely explanation for the invasion. However, *H. irregulare* has a much greater sporulating potential than its congener (33), potentially explaining its much higher transmission rate. Additionally, pure oak stands have been found to be effectively colonized, maybe saprotrophically, only by the invasive species (44). This ability, never reported for either species, is a likely result of the adaptation of the North American species to its new range and may allow the pathogen to reach pine stands in the highly fragmented landscape of central Italy. One surprising finding that may allow to further our understanding of the genetics of fungal invasions, is the massive hybridization rates observed between the two species. On average, 25% of isolates appears to be an admixture of the two genomes, but in some sites recently colonized, that percentage is effectively over 40% (40). Hybridization has lead to genotypes containing alleles of both species, but when the sequence of 11 loci was analyzed for 30 genotypes, 17% of intragenic sequences were novel chimeric alleles containing portions of sequence of both species (40). This high frequency of intragenic recombination has never been reported for the fungi, and indicates an accelerated evolutionary process is currently ongoing in the zone of sympatry.

6. Diagnosis and management

Symptoms caused by *Heterobasidion* spp. vary depending on the pathogen species involved, the tree species infected, the age and previous use of the forest stand, the soil type, local climate, and possibly atmospheric pollution. In general, *H. annosum* s.s. and *H. irregulare* are more effective than other species within the complex in colonizing the cambial layer and sapwood of their hosts (Table 1), both in the root system and at or just above the root collar. As a result, tree mortality occurs more promptly when these two species are involved. Normally trees will die in clusters and mortality progresses over time spreading outwards in concentric rings emanating from the stump or tree that represented the original infection site. Infection by species other than *H. annosum* s.s. and *H. irregulare* is normally limited to the wood. When fungal colonization occurs in the heartwood (as for instance in the vast majority of infected Norway spruce in Europe), outer symptoms are almost nil, and infection is normally detected upon wind-caused failures or when trees are harvested. When infection occurs in the sapwood (as commonly observed on true fir species in North America), a reduction in the functional sapwood will result in stunted growth, shorter needle retention, and chlorosis, compared to uninfected trees in the same site. However, symptoms will occur only after a significant percentage of the sapwood of the tree has been decayed (48).

Although *Heterobasidion* species are all known to cause a white rot, chemicals secreted by both host and pathogen in the early stages of infection result in a darker, almost purple, stain in the wood. Decay transitions into a dark and “wet” appearance, before assuming its final white rot appearance, often displaying lamination (e.g. separation of the growth rings) and pitting (spotted “bleaching” of the wood due to oxidative processes). Decay can be detected in primary roots, at the root crown, and in the bole, sometimes several meters away from the ground (136). Smaller roots and affected cambial portion of the stem often do not decay in resinous species, but solidify due to impregnation with resin during the initial response of the plant to infection. When establishment of the fungus is on stumps (i.e. primary infection), the top 2-4 cm of the stump surface are never decayed, and decay occurs only under this top layer. When infected trees are logged, decay pockets in the sapwood or a column of heartwood decay are often visible on the top of the remaining stump. When wood colonized by *H. annosum* s.l. is incubated in a moist chamber, the conidiophores of its imperfect state [*Spiniger meineckellus* (Olson) Stalpers] are readily formed and provide a useful aid for diagnosis.

Although relatively rarely, *Heterobasidion* will form a mycelial mat in advanced decayed wood or on the top of ectotrophically colonized roots in some types of soil (136). In general though, a definitive field diagnosis of *Heterobasidion* requires the identification of a fruitbody. Fruitbody formation is different among *Heterobasidion* species and is driven by the ecology of the site (100). In mesic regions, fruitbodies can be formed at or right under the duff layer at the base of infected trees or stumps, while in drier regions fruitbodies are normally under loose bark in the roots or in decay pockets. While basidiocarps can reach the significant size of 40 cm (48), they can also be rather small (1 to few cm) and resemble popcorns (100). In mesic sites, and in the presence of significant infestations, it is often possible to see fruitbodies directly emerging from infected roots and almost appearing as growing directly out of the soil, or circling the entire base of dead seedlings and saplings. Morphological differences among basidiocarps produced by different species are present, but they are small and morphological characters are often overlapping (84). It is advisable to use one of the several DNA-based detection methods to differentiate among species (84, 89). Alternatively the species can be determined by performing in vitro mating tests using known testers of each species (67, 84, 131).

Diseases caused by *H. annosum* s.l. may be referred to as “diseases of the site” because the fungus may remain active in dead stumps and in the root systems for decades (49), thus persisting from one rotation to the next. While there is little chance to eradicate the fungus completely once it is successfully established in a stand, its peculiar infection biology that includes primary infections through airborne spores and a considerable level of host preference of each *Heterobasidion* species, has allowed for the development of preventative control strategies which are amongst the most effective and sustainable in forestry.

Stump removal, including careful removal of all roots, is an effective control strategy against *Heterobasidion* root and butt rots (13, 132), and it is deemed the only means by which adequate control can be accomplished in certain heavily infested sites (36). However, it is an expensive and time-consuming control method that requires the use of machines, making it unsuitable for most forest stands. Furthermore, stump removal may lead to several negative environmental consequences (142). Residual biomass consisting of broken roots smaller than 5 cm are not deemed to represent a significant source of viable inoculum as they are predicted to decompose rapidly (96). Uprooting of all infected trees and at least one row of healthy trees combined with digging 150 cm deep trenches was effective against *Heterobasidion* root rots on pines and not prohibitive in terms of costs in a recreation site in southern California (66). However, digging trenches may injury roots, making them susceptible to *Heterobasidion* airborne infections (70).

While the above strategies are seldom used in practical forestry, measures aimed at preventing or limiting airborne infections are more effective and sustainable. During logging, infection courts are created in the form of wounds and stumps, hence thinning and logging operations should be allocated to periods characterized by low risk of spore infection, which vary depending on the general climate of the area. For example, in northern Europe this imply logging operations to be performed in the wintertime, when temperature is below 0°C (6). In the Alps, *H. annosum* s.l. would be successfully controlled by planning logging in winter, most of spring and early summer (38, 42), while in southeastern USA logging should be performed during the summer, when temperatures reach 40°C thus inhibiting spore germination (25, 122).

When logging is operated in periods of sporulation, stump surfaces should be immediately treated with chemical or biological control agents (108, 119). Sodium tetraborate decahydrate (borax), Disodium OctaBorate (DOT), urea, and the biological control fungus *Phlebiopsis gigantea* (Fr.) Jülich have all proved to be effective (71, 88, 93, 108), and are widely used in practical forestry within manual or mechanized stump treatment programs. Modes of action of control agents were reported or reviewed previously, and include direct effects of borates on fungal metabolism (78), a temporary increase in pH of the stump surface resulting in the inhibition of spore germination following urea treatment (61), and competition for the substrate and hyphal interference when *P. gigantea* is applied (55). *Phlebiopsis gigantea* is currently available in three different formulations across Europe (109), however these products, as well as the above chemical ones, are not registered as pesticides in all countries, and consequently they cannot be used everywhere.

Integrated disease management systems combining different approaches to fight *Heterobasidion* root and butt rots are generally more effective and even cheaper than single control methods (46). Disease control may be achieved by adopting one or all of the following strategies, including: i) the adoption of the widest possible spacing among planted trees to lower the probability of root contacts, ii) delaying thinnings, and, iii) reducing rotation length (70). A long-term option to curb the impact of *Heterobasidion* species is to change tree species composition, favoring species that may be less susceptible to the *Heterobasidion* species locally present (Table 1) (46, 146). This strategy may not completely eliminate the pathogen after a single rotation (82), but should significantly and progressively reduce disease incidence.

Summary Points

1. *Heterobasidion annosum* sensu lato (s.l.) is a species complex comprising five sympatrically and allopatrically differentiated species widely distributed in coniferous forests of the northern hemisphere, each characterized by distinct host preference.

2. All species within the complex have a mixed infection biology: primary infections by spores landing on stump surfaces or wounds followed by secondary infections by vegetative growth of mycelium through root contacts or grafts.

3. Population studies in diseased stands have highlighted that primary infections are key in the epidemiology of *H. annosum* s.l.*,* while secondary infections are less prevalent than in other root diseases such as *Armillaria* spp. or *Phellinus weirii.*

4. Although spores of *H. annosum* s.l. have been reported to travel long distances, there is an increasing evidence of a geographical limited range of effective, viable, dispersal of *H. annosum* s.l. spores.

5. Temporal patterns of availability and abundance of viable airborne inoculum and risk of primary infections vary greatly among forests in different climatic zones.

6. *Heterobasidion* root and butt rots are influenced by a range of factors, including stand age, site location, soil, site history, and forest management.

7. *H. annosum* s.l. may be transported short and long distances in infected wood, become invasive in novel ecosystems, and threaten naïve forests. Its success may be linked to its ability to switch from a saprotrophic to a necrotrophic life style, adopting a trade-off strategy that has proven very successful both from an ecological and evolutionary perspective.

8. While there is little chance to eradicate the fungus completely once it is established in a stand, its peculiar infection biology and epidemiology allow for its control thanks to preventative control strategies aimed at curbing stump infections and tree-to-tree contagion.

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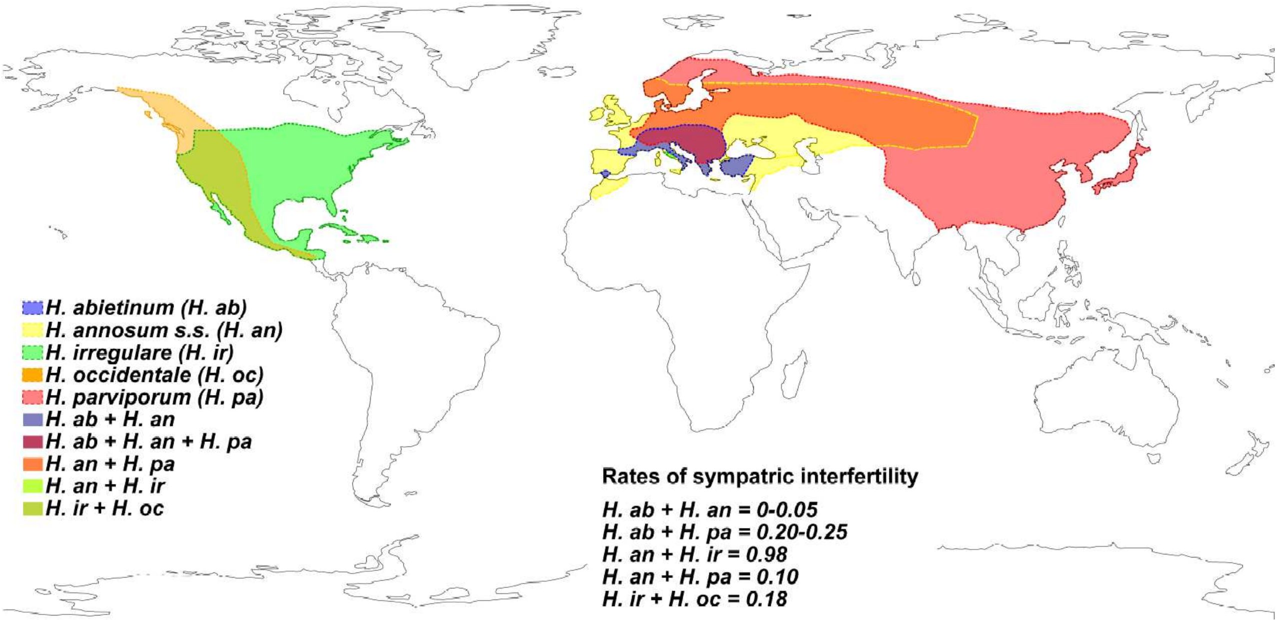
Figures

Figure 1. Worldwide distribution and rate of sympatric interfertility of *Heterobasidion annosum* s.l. species. Distribution map based on previous reports (17–18, 26, 45, 68–69, 100, 146). Rates of interfertility in sympatry based on previously published data (7, 17, 20, 34, 53, 72, 74, 92, 134).



Tables

Table 1 Tentative susceptibility of some tree species to species of *Heterobasidion annosum* s.l. and main host tissue colonized by the pathogen. Table adapted and implemented from Gonthier and Thor (46), with permission of CAB International

	Main plant tissue colonized	*H. abietinum*	*H. annosum* s.s.	*H. parviporum*	*H. irregulare*	*H. occidentale*
Eurasia						
Abies alba Mill.	Heartwood[a]	+++[b]	+	0	-	-
Abies sibirica Ledeb.	Heartwood	-	0	+++	-	-
Larix decidua Mill.	Heartwood	+	+++	++	-	-
Picea abies (L.) Karst.	Heartwood	0	++	++++	-	-
Pinus cembra L. / *P. sibirica* Du Tour	Heartwood	0	+	++	-	-
Pinus pinea L.	Cambium	0	++	0	++[c]	-
Pinus sylvestris L.	Cambium	0	+++	0	-	-
North America						
Abies concolor	Heartwood/Sa	-	-	-	0	+++

(Gord. & Glend.) Lindl. ex Hildebr.	pwood					
Abies grandis (Douglas ex D. Don) Lindl.	Heartwood/Sapwood	-	-	-	0	+++
Calocedrus decurrens (Torr.) Florin	Sapwood	-	-	-	+++	+
Juniperus spp.	Cambium	-	-	-	++++	++
Picea sitchensis (Bong.) Carrière	Heartwood	-	++++[c]	-	0	++++
Pinus elliottii Engelm.	Cambium	-	-	-	++++	+
Pinus jeffreyi Balf.	Cambium	-	-	-	++(+)	+
Pinus ponderosa Lawson & C. Lawson	Cambium	-	-	-	+++	+
Pinus radiata D. Don	Cambium	-	-	-	+	+
Pinus resinosa	Cambium	-	-	-	+++	+

Aiton						
Pinus taeda L.	Cambium	-	-	-	++++	+
Pseudotsuga menziesii (Mirb.) Franco	Heartwood/Sapwood	+[c]	+++[c]	-	+	++
Sequoiadendron giganteum (Lindl.) J. Buchholz	Sapwood	-	-	-	0	++
Thuja plicata Donn ex D. Don	Heartwood	-	-	-	0	++
Tsuga heterophylla (Raf.) Sarg.	Heartwood/Sapwood	-	-	-	0	++++
Broadleaves	Heartwood/?	0	+[d]	0	+?[c]	0

[a] Sapwood instead of heartwood may be colonized in some provenances of *Abies* of the Mediterranean region.

[b] Susceptibility: ++++ = severely damaged; +++ = moderately damaged; ++ = seldom damaged; + = rarely reported; 0 = not damaged (immune).

[c] Allopatric host-pathogen combination.

[d] mostly *Betula* spp., *Fagus* spp., *Populus* spp.