# An integrated approach to control the introduced forest pathogen *Heterobasidion irregulare* in Europe

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The North American fungal pathogen *Heterobasidion irregulare* is currently distributed in pine and oak stands along 103 km of coastline west of Rome, Italy. This paper reviews and expands the knowledge on impacts, pathways of introduction and invasion, factors driving the invasion and on the dispersal abilities of this pathogen in Italy. Further, an integrated disease management program to minimize the spread of the fungus in Europe is suggested, based both on the published literature and on new findings reported here. Observational and genetic evidence support a single introduction through infected wood during World War II, and a subsequent invasion through spore dispersal. Experimental evidence suggests transmission potential of the pathogen rather than hyper-susceptibility of native hosts is the major determinant of invasion. The current range of *H. irregulare* is too vast to suggest eradication, however, we recommend minimizing the risk of spread of *H. irregulare* outside the zone of infestation while reducing the magnitude of infestations within its current range. We provide evidence suggesting the most cost-effective management approach hinges on preventing the saprobic establishment of the fungus in stumps in a 'buffer' area surrounding the current zone of infestation.

## Introduction

Emerging infectious diseases (EIDs) of forest trees are often caused by non-native fungal or fungal-like pathogens accidentally introduced into new areas (Liebhold *et al.*, 2012; Pautasso, 2013; Santini *et al.*, 2013). Several non-native pathogens have had destructive effects on forests over the last century, with consequences not only limited to timber production but encompassing the full range of ecosystem services provided by trees (Boyd *et al.*, 2013). 'Infamous' historical examples include the chestnut blight fungus *Cryphonectria parasitica* (Murrill) M.E. Barr, the white pine blister rust agent *Cronartium ribicola* J.C. Fisch., and the Dutch elm disease pathogens *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, while current emergencies include the sudden oak death pathogen *Phytophthora ramorum* Werres, De Cock & Man in't Veld and the fungus *Hymenoscyphus pseudoalbidus* Queloz, Grünig, Berndt, T. Kowalski, T.N. Sieber & Holdenr. associated with European ash dieback (Maloy, 1997; Fisher *et al.*, 2012; Gross *et al.*, 2014). Since non-native forest pathogens are primarily introduced through the movement of a wide range of substrates including living plants, wood, bark,

seeds and soil (Liebhold *et al.*, 2012; Santini *et al.*, 2013), the number of EIDs is expected to increase as a consequence of the intensification of international trade (Stenlid *et al.*, 2011).

Predicting the locations of pathogen introductions is crucial to prevent new EIDs (Pautasso, 2013). The complexity of the issue is, however, daunting, especially considering that many agents may not be causing any significant diseases in their native range (Parker and Gilbert, 2004). Once an introduced pathogen has established itself into a new area, it may become invasive if its transmission rate exceeds its mortality (McCallum, 2008). However, the period during which the invasion process starts is almost impossible to identify, and is normally dubbed 'lag phase' to convey the idea that there is a lag between the start of the invasive process and our ability to detect the invasion (Garbelotto, 2008). As a consequence of such lag phases, EIDs are not usually identified soon enough to allow for successful eradication campaigns (Gonthier and Garbelotto, 2013). The management of EIDs can be difficult once the invasive process has started, and the timing and size of control efforts is crucial (Stenlid *et al.*, 2011). In general, successful control strategies need to integrate efforts at different spatial and temporal scales (Stenlid *et al.*,

2011), as reported for *P. ramorum* in the USA and *P. cinnamomi* Rands in Australia (Rizzo *et al.*, 2005; Cahill *et al.*, 2008).

It has recently been suggested that EID containment practices may be implemented only when the factors driving the invasion are known (Gonthier and Garbelotto, 2013). Although complex and interrelated, the determinants of invasiveness for pathogens may be grouped into two broad categories. The first hinges on the relative susceptibility of native hosts as the primary determinant, while the second hinges on the pathogen's ability to be transmitted from an infected to an uninfected host (Gonthier and Garbelotto, 2013). For instance, if high host susceptibility is driving the invasion, the EID may be placed within the first category, and a successful control strategy may include breeding for resistance, selective removal of more susceptible individuals and planting of more tolerant or resistant ones. If transmission is driving the invasion, the EID may be a better fit for the second category, and a different strategy may be needed, mostly focused on limiting the production of infectious inoculum (Gonthier and Garbelotto, 2013). This may include understanding and limiting the ecological factors and the substrates that are most conducive to sporulation, spread and infection.

The basidiomycete species complex *Heterobasidion annosum* (Fr.) Bref. comprises five species regarded as some of the most destructive pathogens of conifers worldwide (Garbelotto and Gonthier, 2013). Taken together, the three Eurasian *Heterobasidion* species are responsible for the root and butt rot of several conifer species, with losses estimated at 790 millions Euros per year in Europe (Woodward *et al.*, 1998). *Heterobasidion* species infect their hosts by means of airborne spores landing either on freshly cut stumps or fresh wounds (primary infection). Once established, these fungi may infect uninjured trees by growth of the mycelium through root contacts or grafts (secondary infection) (reviewed by Garbelotto and Gonthier, 2013), thus increasing disease incidence without the need for sporulation. The primary and secondary infection pathways described above explain why disease incidence increases significantly in association with modern forestry and plantation practices (Korhonen *et al.*, 1998; Garbelotto and Gonthier, 2013).

*Heterobasidion* species have long been regarded as unlikely introduced pathogens because of the short life span of their airborne spores, their inability to freely grow in the soil, and the lack of resting or survival propagules (Garbelotto and Gonthier, 2013). However, in 2002 fruiting bodies of the North American species *Heterobasidion irregulare* Garbel. & Otrosina were found in mortality centers of Italian stone pine (*Pinus pinea* L.) in the Presidential Estate of Castelporziano, ~20 km south west of Rome (Gonthier *et al.*, 2004). Currently, the known hosts of *H. irregulare* in Italy include Italian stone pine and Aleppo pine (*P. halepensis* Mill.) (Gonthier *et al.*, 2004; Scirè *et al.*, 2008). The fungus has also been reported as a saprobe on stumps of Mediterranean heath (*Erica arborea* L.) (Gonthier, 2006).

Based on circumstantial evidence, it was speculated that *H. irregulare* may have been introduced during World War II by the US Army through crates and other woody implements made of untreated infected wood (Gonthier *et al.*, 2004). Additional surveys revealed the fungus to be present in every Italian stone pine stand on a 103-km-long stretch of Mediterranean coastline west of Rome (D'Amico *et al.*, 2007; Gonthier *et al.*, 2007). While these findings indicated that *H. irregulare* had become invasive, they also challenged the validity of the 'military/wood' hypothesis, which hinged on the fact that the 5900-ha large Castelporziano Estate is entirely circled by a wall and for centuries it has been – and still is – off-limits to all but a few invited guests. The only known exception is that of the 85th division of the US Fifth Army, which breached the wall and set up a camp under the pines of the Estate, in June 1944. As is the case for any invasive non-native plant pathogen, the accurate reconstruction of its introduction pathway(s) and the identification of the original introduction site(s) are both crucial elements necessary to properly predict its rate and mode of spread. Last, this knowledge may have significant implications for the implementation of policies and effective control strategies aimed at limiting the spread of non-native pathogen species with comparable biology at both worldwide and regional scales (Fontaine *et al.*, 2013).

Interestingly, the native Eurasian sister species *H. annosum sensu stricto*, hereafter referred to as *H. annosum*, is also present in the coastal pine stands west of Rome, but at very low levels compared with *H. irregulare* (Gonthier *et al.*, 2007). This observation may suggest that the non-native species has greater fitness than the native sister taxon in the Mediterranean climatic conditions west of Rome (Gonthier *et al.*, 2007). While *H. irregulare* and *H. annosum* diverged in allopatry ~34–41 million years ago (Dalman *et al.*, 2010), they are both pine-associated species, their mating systems have remained largely compatible (Stenlid and Karlsson, 1991), and interspecific hybrid swarms between the two have been reported in the zone of invasion of *H. irregulare* in Italy (Gonthier *et al.*, 2007; Gonthier and Garbelotto, 2011). As it often occurs when studying non-native species, the coexistence in nature of an invasive species and a close native relative is the best scenario to conduct comparative observations and to design experiments aimed at the identification of factors driving the success of the invasive species (Gonthier and Garbelotto, 2013). This is particularly true when the species share a comparable biology and epidemiology, as is the case of *H. irregulare* and *H. annosum*.

This paper reviews and expands the current knowledge on the ecological impacts associated with the invasion, on the pathways of introduction and spread, and on the factors driving the invasion of the North American *H. irregulare* in Italy. Additionally, the paper attempts to provide details on an integrated disease management program to minimize the losses and the risk of further spread of the invasive fungus in Europe. Most of the information here reported, whether published or first presented in this paper, were generated thanks to a multidisciplinary project involving five Universities and/or Research Centers in Italy and abroad.

## Current distribution and impacts of *H. irregulare*

Results of spore trappings performed as previously described (Gonthier *et al.*, 2007) in 30 sites located in the western part of central Italy show that *H. irregulare* is currently distributed in all coastal pine stands, and even in patches of pine spanning from the Fregene Monumental Pinewood (41°51′25.40″N; 12°11′52.79″E) in the north to a small urban stand in San Felice Circeo (41°15′06.68″N; 13°20′13.17″E) in the south (Gonthier *et al.*, 2007). The presence of the pathogen has been documented as far as 9 km inland from the coast at Castel di Guido (41°54′3.51″N; 12°16′55.43″E) in the north and 18 km from the coast at Fossanova (41°27′14.75″N; 13°11′45.09″E) in the south
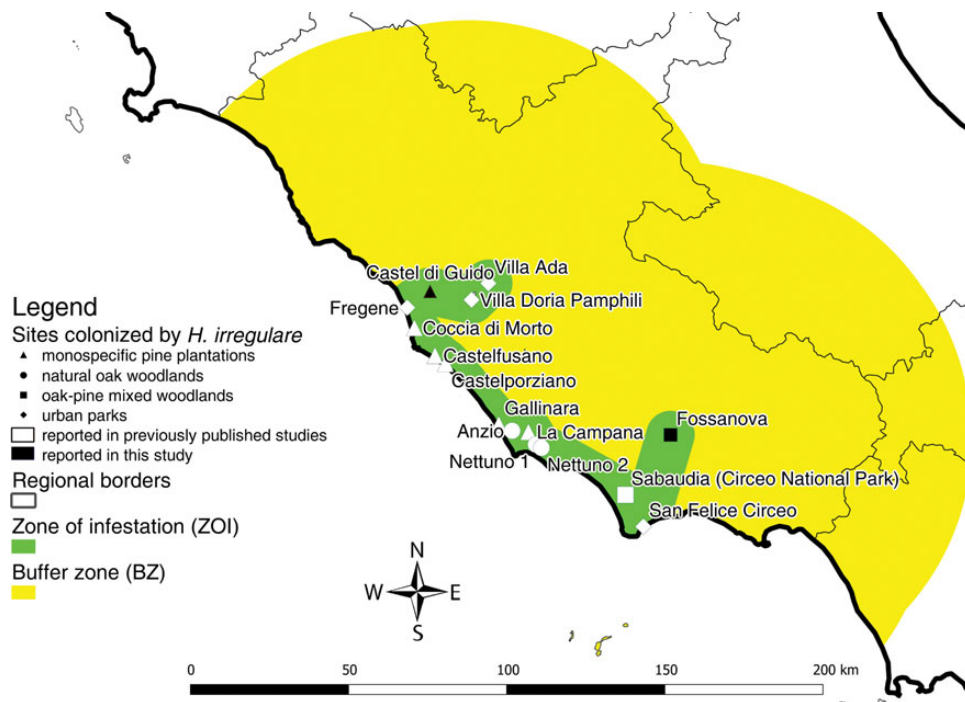
**Figure 1** Current distribution of *Heterobasidion irregulare* in Italy based both on new reports and on previously published data (Gonthier *et al.*, 2004, 2007, 2012; D'Amico *et al.*, 2007; Scirè *et al.*, 2008). The zone of infestation (ZOI) and buffer zone (BZ) in which management prescriptions and options should be implemented to control the disease are shown.

(Figure 1). *Heterobasidion irregulare* was also reported in the city of Rome (D'Amico *et al.*, 2007; Scirè *et al.*, 2008). The pathogen can be found in monospecific pine plantations, in urban parks, in oak-pine mixed woodlands and in natural oak woodlands (Gonthier *et al.*, 2012) (Figure 1). In the gardens of some historical villas in the city of Rome the fungus was reported to be infecting both Italian stone and Aleppo pines (Scirè *et al.*, 2008, 2009), while in pure oak woodlands – where conifers were notably absent – the pathogen was presumed to be mostly a saprobe on oak wood (Gonthier *et al.*, 2012). While *H. irregulare* is reported as a pathogen on both Aleppo and Italian stone pine, significant mortality of groups of trees (upward of 100) has been reported only for the latter species (D'Amico *et al.*, 2007; Gonthier *et al.*, 2007).

Inoculation experiments have confirmed that *H. irregulare* is comparably pathogenic on both Italian stone and Aleppo pines (Scirè *et al.*, 2008, 2011; Garbelotto *et al.*, 2010). The difference in observed mortality rates thus may not be due to higher susceptibility of Italian stone pine, but rather to its abundance on the coast of Latium.

A geographic information system (GIS) analysis of canopy gaps (as a proxy of mortality) performed in monospecific Italian stone pine plantations colonized by *H. irregulare*, through visual photo interpretation of satellite images, revealed that the mean gaps surface in Coccia di Morto (1237 m$^2$) was not significantly different from that in Castelporziano/Castelfusano (2071 m$^2$) ($P > 0.05$), but both were significantly higher than the one recorded in the areas of Anzio and Nettuno (306 m$^2$) (Figure 2). By assuming a 40 years lag between the infestation of Castelporziano/Castelfusano (sites of first introduction) and the area of Anzio and Nettuno (see the section Reconstruction of the invasion below),

the rate of radial enlargement of gaps may be estimated at 40 cm per year.

The invasive pathogen has yet to 'encounter' significant populations of Scots pine (*P. sylvestris* L.), the most widely distributed pine species in central and northern Europe. However, inoculations experiments have shown this species to be rather susceptible to *H. irregulare* (Garbelotto *et al.*, 2010).

Based on previously published data (Gonthier *et al.*, 2007), it appears that infection of Italian stone pines is driven by host density rather than by host population size, as evidenced by the fact that airspora of the invasive species was trapped even in small and isolated clusters of trees. However, no or very low numbers of spores were trapped along pine-lined roadways extending often for tens of km between infested sites (Gonthier P. and Garbelotto M., unpublished data). These two contrasting results suggest most of the damage will continue wherever Italian stone pines are planted in pure stands and follow a regular grid pattern.

The sparse lone Italian stone pines which play key ecological and hydrogeological roles in the Mediterranean maquis and in coastal dune ecosystems, may be less prone to infection. However, it should be emphasized that predictions of damage progression are characterized by high uncertainty, due to possible adaptation and trait evolution of any invasive pathogen (Parker and Gilbert, 2004). For instance, the significant and unexpected presence of the pathogen in pure oak woodlands may lead to unpredictable outcomes. Furthermore, it is unclear what impact the invasive pathogen may have in maquis areas where Italian stone pines grow intermingled with Mediterranean heath and with other confirmed hosts such as junipers (*Juniperus* spp.).
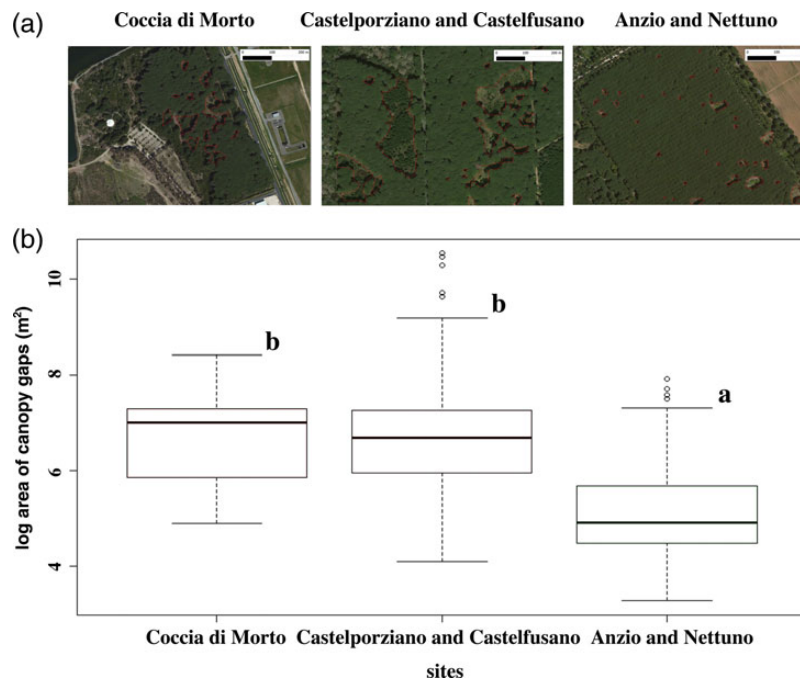
**Figure 2** (a) Canopy gaps in monospecific Italian stone pine plantations of Coccia di Morto, Castelporziano/Castelfusano and Anzio/Nettuno obtained from visual photo interpretation of satellite images. For analyses, data of Castelporziano and Castelfusano and of Anzio and Nettuno were pooled together because of their geographic proximity. (b) Boxplots of the surface of canopy gaps expressed as log transformation of their area in square meter for the above areas. Different letters indicate significant differences in the mean gaps surface ($P < 0.05$; Kruskall–Wallis and Mann–Whitney $U$ test with Holm $P$-value correction).

As timber use of Italian stone pinewood is limited, the mortality caused by *H. irregulare* primarily affects the pine-nut production industry, as well as the extremely important recreational use that these stands offer. Threats are posed by limb failures and tree uprootings, which reportedly can be high in infested sites (Marosy and Parmeter, 1989).

Based on the documented susceptibility of Scots pine to *H. irregulare* and on the effects reported in Scots pine stands infested by *H. annosum* (Sierota, 2013), there is little doubt that an expansion of the zone of invasion out of central Italy would result in significant ecological impacts, potentially ranging from slope instability and increased insect outbreaks in the Alps, to the loss of pine as key timber species and keystone ecological species in central and northern Europe, where it often forms monospecific stands. Potential impacts of *H. irregulare* may include interaction with other native plant pathogens, as shown through inoculation experiments for *Diplodia pinea* (Desm.) J. Kickx f. (Bonello *et al*., 2008).

## Evolutionary consequences of the invasion

The invasion of *H. irregulare* in the area once occupied exclusively by the European species *H. annosum* has fostered massive interspecific hybridization between the two, potentially accelerating the adaptive evolution of the invasive species (Gonthier and Garbelotto, 2011). Based on the results of amplified fragment length polymorphism (AFLP) and sequence analyses, 25 per cent of all isolates studied in the zone of infestation have genomes that are admixtures of the two species, and in sites recently invaded, that percentage may exceed 40 per cent (Gonthier and Garbelotto, 2011). Gene introgression is mostly occurring from the native species into the invasive one, but a low frequency of introgression of *H. irregulare* alleles into *H. annosum* has also been detected (Gonthier and Garbelotto, 2011). When the sequences of 11 loci were analyzed for 30 genotypes, 17 per cent of them identified novel chimeric alleles containing portions of loci from both species (Gonthier and Garbelotto, 2011), suggesting that rapid evolution is ongoing in the zone of sympatry (Garbelotto and Gonthier, 2013). At present, no studies have determined how gene introgression may be affecting fitness and virulence of either the invasive, the native or both species, but examples from other organisms, including several plant pathogens, point to gene introgression as an important evolutionary mechanism, increasing adaptation and pathogenicity of the species involved (Brasier, 2001).

## Reconstruction of the invasion

The results of recent population genetic analyses showing higher allelic richness of the pathogen in Castelporziano/Castelfusano compared with other sites clearly support an introduction in the Castelporziano/Castelfusano area and exclude the possibility of multiple introductions (Garbelotto *et al*., 2013). Based on the maximum number of sequence alleles in 11 unlinked loci and on the results of Bayesian clustering analysis using data from 12 microsatellite loci, three heterokaryotic *H. irregulare* genotypes may have been introduced from a single source population (Garbelotto *et al*., 2013), located in the southeastern USA (Linzer

et al., 2008). Only six *H. irregulare* mating factor alleles were identified in the infested area (D'Amico et al., 2007), and such a finding is also consistent with an introduction of three heterokaryotic genotypes.

As a consequence of the effects of serial bottlenecks, genetic diversity of *H. irregulare* populations decreases with increasing distance from Castelporziano/Castelfusano, supporting an invasion process starting in Castelporziano and ending in the area south of the Circeo National Park (Garbelotto et al., 2013). Two additional observations support this pattern of invasion: (1) disease foci associated with the exotic pathogen were about one order of magnitude larger in Castelporziano than anywhere else, the smallest being in the Circeo National Park and, (2) the large forest of the Circeo National Park was only infested by *H. irregulare* in its northern portion, suggesting recent colonization from the north, where Castelporziano is located (Gonthier et al., 2007, 2012).

The finding that Castelporziano is the most likely single site of the original introduction has great relevance because it makes it extremely plausible that *H. irregulare* may have been introduced into Italy through the movement of infected untreated wood by the military. Besides the obvious worldwide implications for policy-making, this conclusion indicates wood of susceptible hosts should not be moved outside the zone of infestation, unless treated.

## Factors driving the invasion

The co-occurrence of a native and a non-native species sharing similar host preference (pines) and the same infection biology has allowed for comparative observations and experiments aimed at elucidating the relative importance of factors driving the invasion. *Heterobasidion irregulare* introduced to Italy was reported to grow more rapidly than *H. annosum* in growth test performed *in vitro* at temperature ranging from 5 to 30°C (Scirè et al., 2011). However, when genotypes preselected for the absence of alleles introgressed from either species were compared in terms of growth rate no significant differences were observed, at least at temperatures >8°C (Figure 3), suggesting that pure lineages of the two species perform similarly. Likewise, based on the results of comparative inoculation experiments, pathogenicity levels of pure *H. irregulare* and *H. annosum* genotypes are comparable on seedlings or cuttings of several pine species, including Italian stone pine, Scots pine and loblolly pine (*P. taeda* L.), suggesting the invasion is not driven by a disproportionate pathogenicity of the non-native fungus on native pine species (Garbelotto et al., 2010). On the other hand, when inoculated in Scots pine logs, *H. irregulare* genotypes were shown to be able to colonize a volume of wood significantly larger (on average ~5 times larger) than *H. annosum* genotypes (Giordano et al., 2014). Furthermore, several metrics (i.e. the number of fruiting bodies and *PPI*, an index summarizing the amount of surface available for spore production) indicated that saprobic growth was positively correlated with fruiting body production (Giordano et al., 2014). This may provide an explanation for the observation that spore deposition in the infested area was constantly high for the introduced, but not for the native fungal species (Garbelotto et al., 2010). The latter alternated periods of high spore deposition with periods of low spore deposition (Garbelotto et al., 2010).

*Heterobasidion irregulare* is able to colonize pure oak stands that are unavailable to its native sister species (Gonthier et al., 2012),
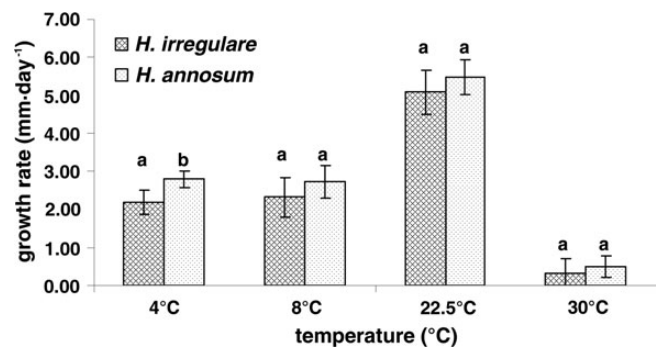


**Figure 3** Comparison of the average *in vitro* growth rates (in mm day$^{-1}$) between 10 genotypes of *Heterobasidion irregulare* and 10 genotypes of *H. annosum* preselected for the absence of alleles introgressed from either species or, for *H. annosum*, isolated at least 100 km far from the invaded area at 4, 8, 22.5 and 30°C. Genotype IDs were: CP2, CP8, CP15, CP18, 4SA, 38NA, 39NE, 45SE, 48NB, 81EE (for *H. irregulare*) and Pd3, Ha carp, J7, CAL1, LG12, 19, 20, 22, 30, 33 (for *H. annosum*). Further information on genotypes is reported in Gonthier et al. (2004, 2007) and Gonthier and Garbelotto (2011). The last five genotypes were isolated by Naldo Anselmi. Data were obtained by measuring at daily interval the mean radial colony growth on malt extract agar medium in 9 cm diameter Petri dishes. Genotype data were obtained by averaging the values of 12 replicates. For each temperature, different letters above bars indicate a significant difference between species ($P < 0.05$; *t*-test). Error bars refer to the standard deviation.

thus broadening the habitats accessible to the non-native pathogen. Although accurate investigations are needed to exclude occurrence of disease caused by *H. irregulare* on oaks, preliminary observations suggest it is present in oak stands only as a saprobe (Gonthier et al., 2012).

Cumulatively, results of observations and experiments suggest that differences in transmission potential, referred to as the pathogen's ability to be transmitted from an infected to an uninfected host, rather than hyper-susceptibility of native hosts may be driving the invasion of the introduced *H. irregulare* in Italy. Such a finding has important implications for disease control.

## Dispersal potential

The rate of spread of *H. irregulare*, calculated by dividing the distance between Castelporziano and the furthest infested site by the time elapsed since introduction, has been estimated at ~1.3 km year$^{-1}$ (Gonthier et al., 2007). Since the movement of Italian stone pine wood, including firewood, in the area is very limited, it is unlikely that the invasion may have been mediated by human transport. The estimated spread rate is obviously an underestimation as it does not take into account that a certain amount of time must have been required for establishment of the introduced species, during the so called 'lag-phase' of the invasion. However, it should be noted that available habitats (forest stands) are extremely fragmented in the infestation area. The analysis of the current distribution of the pathogen and spatial autocorrelation analyses using genetic data may provide a more useful estimate of the true potential dispersal rate of the pathogen and of the effects on its dispersal caused by habitat fragmentation.

**Table 1** Pairwise $\Phi$ST (below diagonal) between populations of *Heterobasidion irregulare* in Italy (from Garbelotto *et al.*, 2013); pairwise gene flow ($M =$ Nm) estimates based upon $\Phi$ST (above diagonal) (this study)

|  | Anzio | Castelfusano | Circeo | Fregene-Coccia di Morto | Nettuno |
|---|---|---|---|---|---|
| Anzio | – | 9.872* | 4.049* | ∞* | ∞* |
| Castelfusano | 0.0482 | – | 4.041 | ∞* | 9.132* |
| Circeo | 0.1099 | 0.2872* | – | 4.041 | 4.262* |
| Fregene-Coccia di Morto | 0.0000 | 0.0000 | 0.1101 | – | ∞ |
| Nettuno | 0.0000 | 0.0519 | 0.1050 | 0.0000 | – |

*P*-value calculated with 10 000 permutations, and marked as significant with an asterisk after Bonferroni correction if $P < 0.0051$. For details on the methods and the dataset refer to Garbelotto *et al.* (2013).

The current distribution of *H. irregulare* appears to be asymmetrical with respect to the putative Castelporziano introduction site. Fregene, the northernmost known infestation site is 24 km north west of Castelporziano, while the southernmost infestation is San Felice Circeo, 79 km south east of Castelporziano (Gonthier *et al.*, 2007). There is a gap of ~50 km between Fregene and the closest significant pine stand northward (Gonthier *et al.*, 2007), while the widest gap between any two adjacent pine stands is only ~30 km in the south. Even taking into account the recent discovery that *H. irregulare* may colonize oak woodlands (Gonthier *et al.*, 2012), the size of gaps north of Fregene remains unchanged, while in the southern zone of infestation the presence of oak forests reduces the widest vegetation gap to ~24 km. Thus, historically *H. irregulare* has been able to cross vegetation gaps up to 20–30 but not 50 km. When comparing migration (*M*) and PHIst values (Table 1; for PHIst see also Garbelotto *et al.*, 2013), it appears that distances up to 10–15 km between sites lead to infinite migration levels and lack of population genetic structure, suggesting strong gene flow within these distances, while for distances of ~20 km *M* was ~9. Note that the infinite migration level between Anzio and Fregene-Coccia di Morto, two sites located at a distance exceeding 20 km, may be an exception and the likely result of historical contemporary migration north (Fregene-Coccia di Morto) and south (Anzio) from Castelporziano of genotypes bearing the same alleles (Hey, 2010). Overall, the difference in *M* values between 10 and 20 km is interesting and suggests a threshold effect on migration when habitat fragmentation exceeds 10–15 km. Additional results from spatial autocorrelation analyses using microsatellite data for Italian populations of *H. irregulare* showed a very significant under-dispersion of alleles up to 500 m, a significant under-dispersion of alleles up to 10 km and a significant over-dispersion of alleles over 80 km (Garbelotto *et al.*, 2013). Although spatial autocorrelation analyses are strongly affected by sampling schemes, it appears rather convincing that the under-dispersion of alleles at distances <10 km may be indicative of the ability of the pathogen to easily cross gaps up to 10 km (Garbelotto *et al.*, 2013).

Research suggests that in regions where available habitats are 500 m–10 km apart (such as the pine stands stretching from Tuscany to northeastern Spain, or the Scots pine plantations of central and northern Europe), the *H. irregulare* spread rate may be much higher than the 1.3 km year$^{-1}$ previously estimated (Gonthier *et al.*, 2007) in the highly fragmented habitats of the current zone of infestation. Thus, the arrival of *H. irregulare* in the less fragmented habitats of central and northern Europe is likely to result in a much faster rate of colonization of the European continent (Garbelotto *et al.*, 2013).

**Table 2** Integrated disease management program to minimize the damages and risk of spread of *H. irregulare* in Europe

| Management action | ZOI[1] | BZ[2] | Prescription/option |
|---|---|---|---|
| Avoidance of logging injuries | X | X | Prescription |
| Careful handling of infected logging residues | X | X | Prescription |
| Changing of tree species | X | – | Option |
| Limitations to the movement of wood and wood products | X | – | Prescription |
| Sanitation and local eradication through destumping | – | X | Prescription |
| Sanitation fellings | X | – | Prescription |
| Stump treatments | X | X | Prescription |
| Trenching and uprooting | X | – | Option |

[1]Zone of infestation: <10 km from confirmed infestation sites.
[2]Buffer zone: >10 km and <90 km from confirmed infestation sites.

## Management guidelines

Given the significant size of the *H. irregulare* infested area, and considering that the pathogen can often be undetected for years (Otrosina and Garbelotto, 2010; Garbelotto and Gonthier, 2013), the complete eradication of the pathogen appears an unrealistic management option, and eradication may be implemented only locally, in the presence of small and isolated foci. Resources should instead be allocated to minimize the risk of spread of the invasive organism outside the current zone of infestation, while reducing infection rates within the infested area (Table 2). Based on the current knowledge of the factors driving the *H. irregulare* invasion, management should mostly aim at preventing the establishment of the fungus during its saprobic phase, thus limiting primary disease transmission. Because the fungus can remain viable for long periods in wood (Gonthier *et al.*, 2004; Garbelotto and Gonthier, 2013; Garbelotto *et al.*, 2013), any management program aimed at preventing further spread must include prescriptions to ensure wood from the zone of infestation is either properly treated before transport, or left on site.

### Zoning of management actions

By combining the information on the distribution of *H. irregulare* in Italy with that on dispersal potential of the pathogen, two zones may be identified (Figure 1). The first one, referred to as the zone of infestation (ZOI), should include not only all known infestations but also all pine and oak stands located within 10 km from any confirmed infestation sites. The second one, referred to as the buffer zone (BZ), should include all pine and oak stands within 80 km from the edges of the ZOI.

### Management prescriptions

#### Stump treatments

The treatment of stump surfaces of conifers and of certain oaks species must be performed in all stands within both the ZOI and the BZ, immediately after felling as previously described (Gonthier and Thor, 2013). Although treatments with both foreign and local strains of *Phlebiopsis gigantea* (Fr.) Jülich were effective against airborne infections of *Heterobasidion* species on Italian stone pine stumps (Capretti and Mugnai, 1988; Annesi *et al.*, 2005), application of urea at 30 per cent concentration may be a preferred initial treatment approach. Urea treatments have in fact been shown to be effective on a wide range of tree species and do not require governmental approval in Italy (Pratt *et al.*, 1998; Nicolotti and Gonthier, 2005). Although still not approved in the EU for use as stump treatments, borates such as sodium tetraborate decahydrate (borax) and disodium octoborate tetrahydrate (DOT) may also be effective against airborne infections, especially on stumps of tree species lacking the urease enzymes needed to hydrolyze urea (Johansson *et al.*, 2002).

Although sporulation of *H. irregulare* appears to be constant throughout the year, it may be beneficial to fell trees during the hottest months, as it is routinely done to control *H. irregulare* in pine plantations of the southern USA (Ross, 1973). Summer felling results in a lower incidence of primary infection, due to a combination of lower inoculum loads and lower percentages of successful infection (Driver and Ginns, 1969).

#### Avoidance of logging injuries

Although wounds on pines may not be as important as infection courts for *Heterobasidion* spp. as they are on other tree species (reviewed by Gonthier and Thor, 2013), prescriptions aimed at avoiding injuries on trees during logging operations may be appropriate in both the ZOI and BZ. Beneficial effects of these prescriptions may include the reduction of the infection risk of other root and butt rot fungi, such as *Phaeolus schweinitzii* (Fr.) Pat. and *Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch.

### Management prescriptions and options in the zone of infestation

#### Sanitation felling

All dead and diseased trees must be felled and removed from the stand as soon as possible. A prompt removal of dead and diseased trees may have the added value of preventing outbreaks of bark beetles such as *Tomicus piniperda* (Linnaeus).

### Trenching and uprooting

To protect high value or highly used recreational sites from the secondary spread of *H. irregulare* through root contacts, isolation trenches 150-cm deep may be excavated around diseased trees as previously described (reviewed by Gonthier and Thor, 2013). The effectiveness of trenching is dependent on the proper siting of the trenches to ensure all infected trees are isolated (Eyles *et al.*, 2008). Thus, trenching needs to include a safety buffer of at least 1–2 rows of completely healthy trees (Kliejunas *et al.*, 2005; Gonthier and Thor, 2013). It has been suggested that trenching, often resulting in root wounds that might be susceptible to airborne infections, could promote the spread of the fungus instead of controlling it (Korhonen *et al.*, 1998). However, trenching combined with uprooting of all trees present within the trench has been reported to be effective in preventing further spread of the pathogen and not prohibitive in terms of costs in both North America and Europe (Kliejunas *et al.*, 2005; Pratt and Wang, 2013).

### Changing of tree species

Replacing pines with less susceptible conifers or with broadleaves (Garbelotto and Gonthier, 2013; Gonthier and Thor, 2013), thus turning a susceptible forest into a theoretically more tolerant one could be an appropriate management strategy in heavily infested sites. Based on the current knowledge, however, this strategy would be more effective in lowering local damage rather than in preventing disease transmission. Furthermore, the finding that *H. irregulare* is also associated with oaks (Gonthier *et al.*, 2012) suggests that replacement of pines with broadleaves may not be sufficient to clean the site from pathogen inoculum.

### Management prescriptions in the buffer zone

#### Sanitation and local eradication

Any tree found to be infected by *H. irregulare* in the BZ must be felled as soon as possible and their stumps removed, including all roots down to a diameter of 5 cm. Smaller roots are predicted to decompose rapidly (Omdal *et al.*, 2001) and thus residual biomass is not deemed to represent a significant source of root disease (Gonthier and Thor, 2013). Although stump removal is an expensive and time-consuming control method, it may be pivotal for preventing further spread of the invasive pathogen, if detected early enough (Pratt, 1998).

### Prescriptions on handling of infected logging residues and on the movement of wood and wood products

#### Handling of infected logging residues

After sanitation felling in the ZOI, logs must be debarked and propped up to avoid contact with moist soil to reduce the risk of formation of *H. irregulare* fruiting bodies (Korhonen *et al.*, 1998). For the same reason, stacking of timber should be avoided. If wood chips are obtained from logging residues in the ZOI these could be left on site or used as fuel. The use of chips for mulching in sites different to that of origin should be avoided. Wood resulting from sanitation operations in the BZ must be burned on site or buried in the closest landfill as soon as possible, possibly after a drying phase (if chipping occurs in a dry and hot season). All

**Table 3** Summary of information on *H. irregulare* in Italy

| Information/trait | Main observations/results | Literature |
|---|---|---|
| Geographic distribution | Along the Tyrrhenian coast, from Fregene in the north to S. Felice Circeo in the south; inland, Castel di Guido in the north and Fossanova in the south; the city of Rome | D'Amico *et al.* (2007); Gonthier *et al.* (2007); this study |
| Impact on the field/extent of mortality | Mortality centers including tens of trees and with diameters >50 m in *Pinus pinea* stands | D'Amico *et al.* (2007); Gonthier *et al.* (2007); this study |
| Host list in the field | *P. pinea* and *P. halepensis* | Gonthier *et al.* (2004); Scirè *et al.* (2008, 2009) |
| Available habitats | Pine stands and oak stands | Gonthier *et al.* (2012) |
| Potential hosts based on pathogenicity tests | *P. sylvestris* | Garbelotto *et al.* (2010) |
| Hybridization with *H. annosum* and subsequent gene introgression | Massive hybridization and gene introgression (possibly of adaptive alleles) is mostly occurring from the native species into the invasive one, but a low frequency of introgression of *H. irregulare* alleles into *H. annosum* has also been detected. Hybridization is leading to the creation of new alleles suggesting that rapid evolution is ongoing | Gonthier *et al.* (2007); Gonthier and Garbelotto (2011) |
| Pathways of introduction and invasion | Support evidence: (1) a single introduction of multiple genotypes in the area of Rome from a single source population located in the southeast of the US, (2) an introduction through infected wood during World War II and (3) a subsequent invasion through spore dispersal | Gonthier *et al.* (2004); Linzer *et al.* (2008); Garbelotto *et al.* (2013) |
| Temperature requirements compared with *H. annosum* | The two species do not differ significantly on temperature requirements for growth at most temperatures | This study |
| Pathogenicity levels on European and North American pines compared with *H. annosum* | Pathogenicity levels of the two species are comparable on seedlings or cuttings of several pine species, including *P. pinea*, *P. sylvestris* and *P. taeda* | Garbelotto *et al.* (2010) |
| Saprobic ability compared with *H. annosum* | *H. irregulare* is able to colonize a volume of pine wood significantly larger (on average ∼5 times larger) than *H. annosum* | Giordano *et al.* (2014) |
| Fruiting ability compared with *H. annosum* | *H. irregulare* exceeds *H. annosum* in a number of parameters used as metrics of fruiting body production | Giordano *et al.* (2014) |
| Sporulation compared with *H. annosum* | Spore deposition in the infested area is constantly high for *H. irregulare* but not for *H. annosum* | Garbelotto *et al.* (2010) |
| Dispersal potential | Rate of spread estimated at 1.3 km year$^{-1}$. Based on spatial autocorrelation analyses of molecular data: a significant under-dispersion of alleles up to 10 km and a significant over-dispersion of alleles over 80 km; such distance thresholds may reveal the real spread potential of the pathogen | Gonthier *et al.* (2007); Garbelotto *et al.* (2013); this study |

these operations should be performed as soon as possible to avoid the wood from becoming a source of inoculum.

*Limitations to the movement of wood and wood products*

Green logs and untreated timber of Italian stone pine, Aleppo pine, and of some oak species must not be moved from the ZOI. The same restrictions must be applied for firewood and other wood products made of untreated pines or oaks.

## Concluding remarks

Comparative observations and experiments focused on the invasive *H. irregulare* (Table 3) and the native *H. annosum* have allowed the identification of traits that may be driving the invasion. In summary, non-native *H. irregulare* does not appear to be more virulent on European pines than its native sister taxon (Garbelotto *et al*., 2010), however, it is a better saprobe (Giordano *et al*., 2014), a constant high sporulator (Garbelotto *et al*., 2010), and may have a broader host range than its European counterpart (Gonthier *et al*., 2012). Experimental evidence indicates that higher saprobic colonization leads to higher production of fruiting bodies (Giordano *et al*., 2014), a process that is bound to enhance the transmission and invasiveness of the non-native pathogen. It should be noted that saprobic stump colonization is almost always a precursor to infection of standing trees, which occurs through stump to tree transmission effected by fungal growth via root contacts (Garbelotto and Gonthier, 2013), hence a greater saprobic potential translates into a greater opportunity for pathogenic infection of standing trees.

The three major findings which have been factored in the design of the integrated disease management approach described above are: (1) the significant ability of *H. irregulare* to establish itself as a saprobe, both on pines and oaks, (2) the current ZOI based on both confirmed infestations and on the range of effective dispersal (10 km) of airborne *H. irregulare* spores, and (3) the size of the BZ based on the presumed distance (80 km) at which effective dispersal of *H. irregulare* is minimized. Buffer zones of smaller size have occasionally been suggested for other plant pathogens based on empirical considerations (Reisenzein and Steffek, 2011), but small BZ may be ineffective as suggested for other forest pathosystems (Rizzo *et al*., 2005). The size of the BZ proposed in this study is a conservative preferred measurement based on the precautionary principle and solidly supported by gene flow calculations (Garbelotto *et al*., 2013). The BZ is larger than the size of gaps (50 km north of Fregene) yet to be crossed by the invasive pathogen. Lack of northward spread may in fact have been conditioned by the lack of appropriate substrates. For instance, age and size of trees is proportionally correlated with infection frequency by wood decay organisms, including *Heterobasidion* species (Garbelotto and Gonthier, 2013), and as pine stands north of Fregene get older and larger, establishment of the invasive pathogen may be easier. Hence, the adoption of a buffer zone of 80 km based on genetic data, is preferable and likely to be more durable than selection of a smaller buffer zone based on historical spread of the organism.

Intensive monitoring efforts should be implemented in the buffer area both in pine and oak stands, and wherever the presence of *H. irregulare* after field observation is suspected. A prompt movement of the actual borders of the ZOI and BZ should occur as soon as the pathogen is detected in new sites. Monitoring could be performed using the wood disk exposure method, consisting of the entrapment of *Heterobasidion* spores present in the air combined with a PCR-based diagnostic assay to distinguish between *H. irregulare* and *H. annosum*, as previously described and extensively used (Gonthier *et al*., 2007). Although spores of *H. irregulare* may be found year-round in central Italy, monitoring efforts could be limited to the autumn or spring periods, when the concentration of *Heterobasidion* spores in the air is highest (Garbelotto *et al*., 2010), and hence the detection of the pathogen more likely. When monitoring is conducted during the autumn or spring periods, the distance at which *Heterobasidion* spores can be entrapped may be as high as 500 m (Gonthier *et al*., 2012), thus minimizing the number of sampling points needed for effective *H. irregulare* detection. Monitoring programs should be performed periodically (every 1 or 2 years) and could stop after 20 years only if no increase in the distribution area of the fungus has been detected in the meanwhile.

In conclusion, it should be noted that the management actions here recommended may be successful in preventing further spread of *H. irregulare*, only if properly enforced through quarantine regulations. These regulations need to be promptly adopted by the Italian Government and by the European and Mediterranean Plant Protection Organization (EPPO).

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None declared.

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