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# Comparing the influences of ecological and evolutionary factors on the successful invasion of a fungal forest pathogen

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**Abstract** The fungal forest pathogen *Heterobasidion annosum* has been introduced from North America into Italy and is now associated with high mortality of Italian stone pines. Due to the presence of a closely related native *H. annosum* taxon, this pathosystem presents an unusual opportunity to test specific ecological and evolutionary factors influencing fungal invasions. Comparative inoculation experiments on Scots pine cuttings and on seedlings of European and North American pines failed to identify significant increased pathogenicity of North American genotypes on European hosts congruent with lack of host-pathogen co-evolution. However, spore trappings indicate that while reproductive potential of native *H. annosum* was significantly reduced in the dry season, that of the invasive taxon was consistently high regardless of season. Ecological differences between the native and exotic taxon may therefore facilitate this invasion. Understanding which factors enhance this emerging forest disease is important both for biotic invasion theory and for disease control.

**Keywords** Co-evolution · Emerging infectious disease · Fungi · *Heterobasidion* · Propagule pressure · Pathogenicity

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## Introduction

Theories explaining why some alien organisms can successfully establish and spread upon introduction to a new environment comprise a rich and expanding literature (Prinzing et al. 2002; Duncan et al. 2003; Colautti et al. 2004; Hawkes 2007). Evolutionary processes have been hypothesized to contribute to successful invasions (Baker 1965; Mayr 1965), and there is increasing evidence that evolutionary factors such as hybridization events and phylogeographic history do in fact influence the outcome of an invasion (Facon et al. 2003; Parker et al. 2003; Lambrinos 2004). Contrary to the classic "invasion paradox" (Sax and Brown 2000), in which native organisms are presumed to be better adapted to their environment than invasive ones, invasive pathogens are often predicted to have higher pathogenicity (here a quantitative term indicating ability of a pathogen to cause different levels of disease) and infectivity (a qualitative term indicating the ability to successfully infect a host) than native pathogens which have

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undergone long-term co-evolution the same host (reviewed in Parker and Gilbert 2004).

Specific hypotheses to explain biological invasions also include many traditionally classified as ecological processes, including escapes from the pressure of natural enemies by an exotic organism, resource availability, and favorable disturbance or environmental factors in the invaded area (Elton 1958; Davis et al. 2000; Mack et al. 2000; Keane and Crawley 2002; Torchin et al. 2003; Blumenthal 2006). Emergent diseases may also be facilitated by ecological factors such as high transmission rates in favorable environments (Parker and Gilbert 2004; Woolhouse et al. 2005) or the ecology of disease vectors (Juliano and Lounibos 2005; Malmstrom et al. 2005; Tatem et al. 2006; Togashi and Shigesada 2006; Despommier et al. 2007).

These two broad classes of mechanisms, evolutionary and ecological, however, are not mutually exclusive. A non-indigenous organism's encounter with new biotic and abiotic conditions can lead to strong selection and rapid interaction of both ecological and evolutionary processes in invaded communities (Sakai et al. 2001; Lambrinos 2004), and recent frameworks for understanding successful invasions explicitly integrate both types of processes (Facon et al. 2006; Hufbauer and Torchin 2007; Fausch 2008). Disentangling multiple influences on invasion success can be difficult, especially when limited information is available on the sequence of biotic and abiotic changes during introduction, establishment and spread of an alien organism (Facon et al. 2006).

There have been few empirical attempts to quantify the relative contributions of both ecological and evolutionary processes to the success of invasive plant pathogens. To quantify the potential mechanisms facilitating invasion, such as relative pathogenicity or environment-dependent fitness, it is useful to have a study system with closely related native and invasive pathogens and closely related respective hosts. This type of comparison is particularly difficult in studies of invasive fungi, as there is little baseline data on native fungal communities (Desprez-Loustau et al. 2007). In fact, fungi are often underrepresented in invasion biology despite the fact that they can spread upon introduction (reviewed in Wingfield et al. 2001; Gilbert 2002; Schwartz et al. 2006) and have documented adverse effects on invaded ecosystems.

The *Pinus* (Coniferophyta, Coniferales, Pinaceae)/*Heterobasidion annosum* (Fr.) Bref. (*Basidiomycota, Russulales, Bondarzewiaceae*) pathosystem offers a case in which an invasive fungal forest pathogen has colonized an area inhabited by a closely related native pathogen, thus affording a rare fungal context for testing theoretical predictions grounded in invasion biology and disease emergence. *Heterobasidion annosum sensu lato* (*s.l.*) is among the most ecologically and economically severe pathogens in northern coniferous forests (Woodward et al. 1998). The North American *H. annosum* P intersterility group (ISG) was introduced in coastal forests of Italy presumably during World War II, where its sister taxon, *H. annosum sensu stricto* (*s.s.*), is present (Gonthier et al. 2004, 2007). Phylogenetic analysis has shown that the North American *H. annosum* P ISG and Eurasian *H. annosum s.s.* operational taxonomic units (OTUs) are sister taxa in a monophyletic clade and that the invasive genotypes likely originated in eastern North America (Linzer et al. 2008). *Heterobasidion annosum* P ISG has subsequently become invasive in Italy: both trappings of airborne meiospores and sampling fungal fruitbodies have shown the invasive pathogen is abundant along approximately 100 km in the Latium region around Rome, while the presence of the native *Heterobasidion* on the coast is limited (D'Amico et al. 2007; Gonthier et al. 2007). Although local single-site replacements of the native by the exotic taxon cannot be excluded, surveys in several coastal pine forests outside of the zone of infestation have confirmed the native pathogen to be overall rare along the shores of the Tyrrhenian sea, thus indicating the exotic taxon occurs in areas only marginally occupied by the native species (Gonthier et al. 2007). Exceptionally, both taxa can be found with equal representation in the mixed oak-pine forest of the Circeo National Park (NP), which is distinctive for its mesic, rather than xeric, flora (Gonthier et al. 2007).

Disease characteristics and epidemiology are similar for the North American *H. annosum* P ISG and Eurasian *H. annosum s.s.* Both OTUs are characterized by a broad host range and a preference for the genus *Pinus*, and in both continents, infection by either taxon leads to colonization of cambial tissue in the roots and root collar resulting in tree death (reviewed in Asiegbu et al. 2005). Transmission is effected by airborne meiospores (basidiospores)

produced in fungal fruitbodies, and these propagules can infect freshly cut stumps and standing live trees through wounds. Localized disease spread can also occur through colonization of roots that are either grafted or in contact, resulting in expanding foci of tree mortality (Korhonen and Stenlid 1998; Asiegbu et al. 2005). Significant levels of mortality, unprecedented for xeric *Pinus pinea* L. (Italian stone pine) forests, have been reported in association with the invasion of the exotic *Heterobasidion* OTU (Gonthier et al. 2007), and North American pathogen genotypes have recently been isolated from Aleppo pine (*Pinus halepensis* P. Mill.) in the same region (Scirè et al. 2008). The primary hosts in the invaded (*P. pinea*) and native area (Loblolly pine, (*P. taeda* L.)) are closely related pines both belonging to the subclade *Pinus* within the *Pinus* genus (Gernandt et al. 2005); this relatedness of both pathogens and hosts represent another benefit offered by this pathosystem for comparative studies of the invasion process.

We designed a series of experiments to test a few factors that may be favoring this emergent disease. Two experiments tested whether lack of co-evolution between the North American pathogen and the Italian hosts could lead to increased pathogenicity and/or infectivity of the invading North American *H. annosum* P ISG relative to native *H. annosum s.s.* In the first experiment, pathogenicity of a significant number of native Eurasian, native North American and invasive North American genotypes was compared on cuttings of a single European host of known susceptibility. Scots pine (*Pinus sylvestris* L.) was selected for this experiment because it is the most widely distributed host species for *H. annosum s.s.* in Europe and parts of Asia (Korhonen et al. 1998). The need to assess the threat posed by the introduced pathogen not only to Mediterranean but to Eurasian ecosystems as a whole was also a determinant for choosing this host. In the second experiment, Eurasian and North American pathogen genotypes were used in a common-garden inoculation study quantifying relative pathogenicity and infectivity to seedlings of North American (*P. taeda*) and Italian (*P. pinea*) hosts. Finally, observational data had suggested the xeric environment in the zone of invasion may be more favorable to the invasive than to the native pathogen (Gonthier et al. 2007). Therefore, a third experiment was designed to compare the propagule pressure of the two fungal taxa during dry and wet periods. Reproductive potential was chosen for this experiment because it is both a measure of pathogen fitness and one of the key variables underlying the development of epidemics.

## Methods

### Experiment 1: Inoculation of Scots pine cuttings

The experiment was performed in two phases: first, a host of intermediate susceptibility was selected, and then a comparative inoculation of pathogen genotypes was performed on this host. In order to identify a host of intermediate susceptibility, the following trial was performed: 12 terminal branches from each of 13 *Pinus sylvestris* trees growing in 3 sites in northwest Italy were excised under water, placed in water buckets and brought to the greenhouse at the University of Turin, where they were placed in 15 ml conical Falcon tubes filled with 10 ml tap water and sealed with Parafilm to avoid water evaporation. The methodology is a modification of that based on inoculation of seedlings, the most widely accepted approach to test pathogenicity of pathogens of roots, stems and branches of woody plants, and has been tested successfully in the *Heterobasidion* as well as in other pathosystems (Dodd et al. 2004; Swedjemark and Karlsson 2006; Garbelotto and Schmidt 2009).

Six cuttings for each tree, of about 20 cm length and 6–8 mm diameter, were inoculated with a local genotype of *H. annosum s.s.* (code n. 13, Table 1) by placing a plug of Malt Extract Agar (MEA; 20 g agar, 20 g glucose, 20 g malt extract, 2 g peptone, 1 l water) from the edge of a 15 day colony in a slit obtained by excising a portion of the bark. Inoculation points were wrapped in parafilm, and the remaining six cuttings for each tree were mock-inoculated using a plug of sterile agar. After inoculation, Falcon tubes were kept at 21°C on a 12 h light/dark cycle, and they were periodically refilled with tap water. Vitality of cuttings and their photosynthetic activity was monitored by taking 10 fluorimetric readings every 3 days through a modulated chlorophyll fluorometer OS1-FL (Opti-Sciences, Hudson, NH). A strong decline in vitality was observed at 45 days post inoculation (data not shown), when the experiment was stopped. Each stem was sectioned in rounds 3 mm-thick, which

**Table 1** *Heterobasidion* genotypes used for cutting and seedling inoculation assays

| Genotype code | Isolate | Isolation date | Host of origin | Geographic origin | Lat-long coordinates | *Heterobasidion* taxon[a] | Collector[b] |
|---|---|---|---|---|---|---|---|
| 1 | 365 | ? | *Pinus palustris* | Savannah River, SC, US | 32°50′46″N; 81°32′33″W | *H. annosum* P ISG | WO |
| 2 | 24086C | 2003 | *Pinus taeda* | Phenix City, AL, US | 32°28′22″N; 85°1′12″W | *H. annosum* P ISG | WO |
| 3 | 1116-1 | 2003 | *Pinus taeda* | Phenix City, AL, US | 32°28′22″N; 85°1′12″W | *H. annosum* P ISG | WO |
| 4 | 11063B | 2003 | *Pinus taeda* | Phenix City, AL, US | 32°28′22″N; 85°1′12″W | *H. annosum* P ISG | WO |
| 5 | Conk1 | 2003 | *Pinus taeda* | De Soto National Forest, MS, US | 30°36′8″N; 89° 7′53″W | *H. annosum* P ISG | WO |
| 6 | Conk2 | 2003 | *Pinus taeda* | De Soto National Forest, MS, US | 30°36′8″N; 89° 7′53″W | *H. annosum* P ISG | WO |
| 7 | 1A | ? | *Pinus taeda* | LA, US | 31°38′30″N; 93°19′12″W | *H. annosum* P ISG | NH |
| 8 | 12023B | 2003 | *Pinus taeda* | Phenix City, AL, US | 32°28′22″N; 85°1′12″W | *H. annosum* P ISG | WO |
| 9 | P9P | 1997 | *Picea abies* stump | Charvensod, AO, Italy | 45°41′25″N; 7°19′03″E | *H. annosum s.s.* | PG |
| 10 | P12P | 1997 | *Picea abies* stump | Charvensod, AO, Italy | 45°41′26″N; 7°19′04″E | *H. annosum s.s.* | PG |
| 11 | 4N1052/C | ? | *Pinus sylvestris* tree | Dept. Cote-d′Or, France | 47°31′26″N; 4°37′07″E | *H. annosum s.s.* | SR |
| 12 | E 18.11.3 | 2003 | *Picea abies* stand | Co. Fermanagh, N. Ireland, UK | 45°41′26″N; 7°19′04″W | *H. annosum s.s.* | WB |
| 13 | Pd3 | 1999 | *Pinus sylvestris* tree | Meugliano, TO, Italy | 54°33′07″N; 7°43′31″E | *H. annosum s.s.* | GN |
| 14 | CAL1 | 1995 | *Fagus sylvatica* | Taverna, CZ, Italy | 39°00′57″N; 16°34′48″E | *H. annosum s.s.* | NL |
| 15 | CAL2 | 2002 | *Pinus nigra* spp. *calabrica* | Rogliano, CS, Italy | 39°09′50″N; 16°19′28″E | *H. annosum s.s.* | NL |
| 16 | Cembro1b | 1998 | *Pinus cembra* tree | Cogne, AO, Italy | 45°36′15″N; 7°21′50″E | *H. annosum s.s.* | PG |
| 17 | J41R | 1998 | Airspora in *Abies alba*/ *Picea abies* stand | Jovençan, AO, Italy | 45°42′18″N; 7°16′52″E | *H. annosum s.s.* | PG |
| 18 | 203011 | 2001 | *Pinus sylvestris* tree | Gryfino, Poland | 53°12′00″N; 14°34′00″E | *H. annosum s.s.* | PL |
| 19 | CP0 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | PG |
| 20 | CP2 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 21 | CP3 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 22 | CP7 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 23 | CP9 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 24 | CP12 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |

**Table 1** continued

| Genotype code | Isolate | Isolation date | Host of origin | Geographic origin | Lat-long coordinates | *Heterobasidion* taxon[a] | Collector[b] |
|---|---|---|---|---|---|---|---|
| 25 | CP15 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 26 | CP18 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |
| 27 | CP20 | 2002 | *Pinus pinea* stump | Castelporziano, Italy | 41°42′46″N; 12°24′03″E | *H. annosum* P ISG | NA |

[a] *H. annosum* P ISG = North American *H. annosum* P intersterility group; *H. annosum s.s.* = Eurasian *Heterobasidion annosum sensu stricto*

[b] Collectors, *NA* Naldo Anselmi, *WB* William Bodles, *NH* Nolan Hess, *PG* Paolo Gonthier, *PL* Piotr Łakomy, *NL* Nicola Luisi, *GN* Giovanni Nicolotti, *WO* William Otrosina, *SR* Sylvie Rose

were plated on MEA medium: extent of fungal colonization of each stem was inferred by the number of stem sections found to be colonized by the pathogen as determined by the visible growth of the fungus on the stem section and surrounding media.

A *P. sylvestris* tree that displayed intermediately sized lesions (data not shown) was selected to conduct the comparative inoculation experiment. Using the same methodology described above, a total of 27 fungal isolates, including 8 North American, 10 Eurasian and 9 invasive North American genotypes (Table 1) were each inoculated on 6 cuttings of this single tree. All genotypes of North American provenance were isolated from the most likely source population for the Italian invasion (Linzer et al. 2008). Six cuttings were mock-inoculated as previously described. Fluorimetric readings indicated vitality of branches was rapidly declining at 45 days post inoculation, hence the experiment was stopped, and branches were sectioned. Slices approximately 3 mm in thickness were incubated at room temperature in Petri dishes lined with autoclaved filter paper dampened with sterile distilled water. After 5 days, plates were moved to 4°C for 48 h, then returned to room temperature for 5 more days to induce sporulation. Extent of fungal colonization was determined by microscopic observation of the number of disks exhibiting characteristic *Heterobasidion* conidiophores (Swedjemark and Karlsson 2006).

## Experiment 2: Common garden experiment

Results from the first experiment were used to select a total of 7 pathogen genotypes representative of a range of pathogenicity for use in Experiment 2, including 4 North American and 3 Eurasian genotypes (codes n. 1, 5, 7, 15, 16, 18, 25; Table 1). During preparation for the experiment, a native North American genotype selected for its low-pathogenicity (code n. 5) was discovered to have anomolous growth and re-cultured from the culture collection. Four-year-old seedlings of the European *P. pinea* (Ponto Nursery Inc. Vista, CA 92085; seed source: Italy) and North American *P. taeda* (International Forest Company Moultrie, GA 31768; seed source: coastal South Carolina) were re-potted in conical containers and tended in the UC Berkeley greenhouse for 6 months before being inoculated in April of 2006. The experimental layout included 8 randomized blocks, four for each species. Individual seedlings cannot be randomized across species because their different height and shape makes it extremely difficult to evaluate the development of symptoms on each individual plant. Each fungal genotype was inoculated on 60 seedlings of each of the two hosts: this balance of seven pathogen genotypes and relatively high seedling replication was selected due to the high intra-isolate variability in lesion length observed in our inoculation of Scots pine cuttings and limitations on total experimental seedling number. Inoculation was performed using *Heterobasidion*-colonized pine wedges inserted underbark, as described in Garbelotto et al. (2007). Fourteen *P. pinea* and 23 *P. taeda* negative-control seedlings were mock-inoculated with identically prepared, but uncolonized, wedges. The mean height of experimental seedlings was 51.4 ± 0.06 cm for *P. taeda* and 57.0 ± 0.03 cm for *P. pinea* (mean ± SE). Mean seedling diameter at

inoculation point was 0.785 ± 0.00124 cm for *P. taeda* and 0.970 ± 0.000735 cm for *P. pinea* (mean ± SE).

Any seedlings that died within the first 14 days of the trial were removed from the experiment, as death was unlikely to be due to a direct effect of the pathogen. After 2 weeks and every 14 days thereafter, recently dead seedlings were recorded and sectioned in 3 mm slices. For harvests taking place before the conclusion of the experiment, one *P. taeda* and one *P. pinea* mock-inoculated seedlings were sectioned and incubated at each harvest as negative controls. Slices were incubated and examined to determine extent of fungal colonization, e.g. lesion length, as described for the previous experiment. After 8 months, all remaining living seedlings, including mock-inoculated negative-control seedlings, were sectioned and scored as above.

### Experiment 3: Quantifying the airspora of *Heterobasidion* spp.

The third experiment was performed in the mesic Circeo National Park forest, previously shown to harbor both pathogen OTUs in approximately equal amounts (Gonthier et al. 2007). Although the Circeo NP is on the Mediterranean coast, its pines have been planted on reclaimed swamps since the 1920s; thus this site is ecologically distinct from the other coastal sites, being characterized by a distinctive mesic, rather than xeric, flora. Four sampling points were selected in the forest, based on data from a previous survey (Gonthier et al. 2007). Sampling points were selected where airspora from both OTUs were present in high and comparable amounts in the wet season, a period known to be the favorable to sporulation by most fungi and in particular by *Heterobasidion* (James and Cobb 1984; Fitt et al. 1989; Gonthier et al. 2005; Morales et al. 2006). This sampling scheme minimized potential bias of sampling near fruitbodies of only one of the two OTUs. Propagules were sampled at each point by placing four woody spore traps 5 m from the central sampling point along the four main cardinal directions, as in Gonthier et al. (2007). Traps were consisted of wood disks, 11–12 cm diameter, obtained from Norway spruce (*Picea abies* (L.) Karst.). Wood of Norway spruce has previously been shown to be unselective for the saprotrophic growth of *Heterobasidion* spores and has been extensively used to sample spores of North American and European *Heterobasidion* species (Gonthier et al. 2005, 2007). Spore trappings were conducted at the following times: December 2005, September 2006, December 2006 and September 2007. Traps were left exposed for 24 h at each of the four sampling dates, and emerging colonies of *Heterobasidion* were identified through microscopic observation of traps incubated in moist chambers and repeatedly checked at 1 week intervals for the 4 weeks after trap exposure. Up to 15 randomly chosen colonies per trap were isolated. A similar experiment was performed at the same time in Castelfusano, 80 km north of the Circeo NP, an area in which only the North American taxon is normally detected.

Taxon identification was done by DNA-based molecular diagnosis of isolates, as described in Gonthier et al. (2007). The PCR-based method hinges on DNA primers that will specifically amplify only one of the two OTUs, resulting in differently sized amplicons depending on the taxon analyzed. Diagnosis was conducted using two loci, one nuclear and one mitochondrial. For each of the two pathogen OTUs thus identified, we calculated infection frequency (IF) based on number of traps with at least one positive isolation and deposition rates (DR) based on number of spores trapped averaged over a square meter. We assumed that each colony resulted from deposition of one viable spore (Gonthier et al. 2005). The total relative abundance of spores of each OTU at any given sampling point was assumed to be the same as that determined for the randomly selected subset isolated and analyzed by PCR assays at that same point.

### Analyses

To analyze lesion length data from the inoculation of Scots pine cuttings, we used a univariate general linear model with genotype as a nested random factor within groups (i.e., *H. annosum s.s.*, *H. annosum* P ISG, *H. annosum* P ISG introduced in Italy). Variation within groups was analyzed by a *T*-test comparing the best-performing with the worst-performing isolates of each group.

For the common garden experiment employing inoculations of both Loblolly and Italian stone pines, analyses were performed in R v.2.6.1 (R Development

Core Team 2007) and JMP v 5.0.1 (SAS Institute). One-way analysis of variance (ANOVA) was employed to compare mean length of stem colonized across genotypes on each host species, a measure of pathogenicity. Post hoc Tukey's HSD Tests were used to check for significant differences in mean lesion length between isolates. Logistic regression analyses were used to compare infectivity [infected (1) vs not infected (0)] and mortality [survived (1) vs dead (0)] among fungal genotypes. Spearman's rank correlation was used to examine correlation between host species for two variables (1) length of stem colonized (a measure of pathogenicity) and (2) proportion seedlings infected by each pathogen genotype (a measure of infectivity).

In the experiment aimed at comparing airspora for each *Heterobasidion* species in different seasons, both the Kruskal–Wallis test, with data from sampling points as replicates, and the $\chi^2$ test were used to compare IF values from different collection periods. The Kruskal–Wallis test was also used to compare DR values from different collection periods. The Pearson $\chi^2$ in contingency tables and a 2-tail Fisher's Exact test were calculated to compare propagule pressure, both IF and DR, of the two *Heterobasidion* species in winter (December) and in summer (September).

Gonthier et al. (2005) have shown that the strongest correlation between metereological conditions and sporulation of *Heterobasidion* spp. is found when considering the 4 week long period preceding sampling. Thus, meteorological conditions of the 4 week period preceding each sampling described in this study were characterized based on the daily data collected at the meteorological station of the Latina airport (41°33′0″N, 12°54′0″E, 26 m a.s.l.). Data from the two winter (December) and summer (September) samplings were averaged to determine individual values representative of each of the two seasons over the course of our 2 year long experiment. The Mann–Whitney *U* test with a significant cut-off of 5% was used to compare winter and summer mean temperature (°C), mean daily precipitation (mm day$^{-1}$), and De Martonne aridity Index (*I*) values. The aridity Index *I* (De Martonne 1926) was calculated as $I = P/(T + 10)$, where *P* is the precipitation in mm and *T* is the mean temperature in °C; this index summarizes drought conditions and has successfully been used to describe aridity on a monthly scale (Čufar et al. 2008).

## Results

### Lesions on scots pine cuttings

Significant variability in lesion size was observed among genotypes within the North American and Eurasian taxa (*T*-value = 7.3209, d.f. = 10, $P < 0.0001$; *T*-value = 2.6075, d.f. = 10, $P = 0.0262$, respectively) but not within the invasive North American genotypes from Italy (*T*-value = 2.0061, d.f. = 10, $P = 0.0726$) (Fig. 1). When performing the overall analysis comparing the three groups, there were no significant differences among groups or genotypes (Table 2).

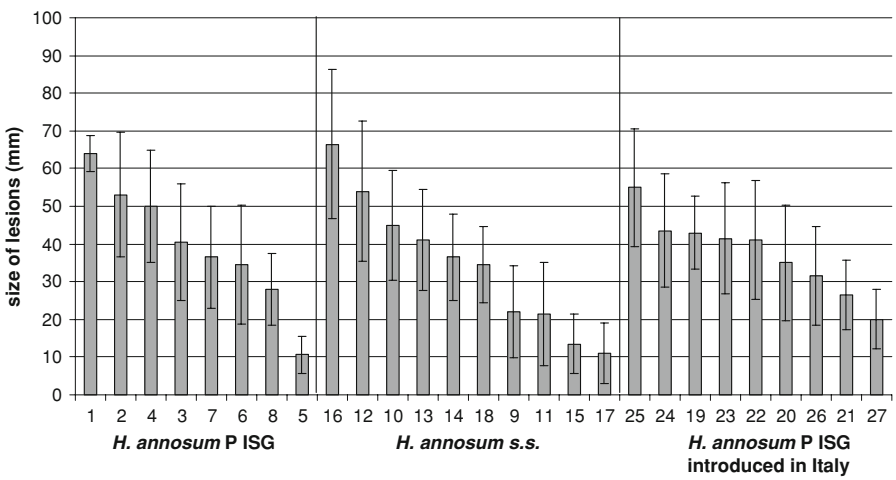


**Fig. 1** Size of lesions caused by 8 North American (*H. annosum* P ISG), 10 Eurasian (*H. annosum sensu stricto*) and 9 North American *Heterobasidion* genotypes introduced in Italy in cuttings of one genotype of *Pinus sylvestris* 45 days after inoculations. *Bars* show standard errors (SE). See Table 1 for genotype codes

**Table 2** Summary of the univariate general linear model for the comparison of the three groups in terms of lesion length in Scots pine cuttings

| Source | | Sum of squares; type III | *df* | Mean squares | *F* | *P* |
|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 34257.639 | 1 | 34257.639 | 29.556 | 0.000 |
| | Error | 136860.658 | 118.077 | 1159.076 | | |
| Groups | Hypothesis | 2150.431 | 3 | 716.810 | 0.546 | 0.654 |
| | Error | 51351.614 | 39.108 | 1313.069 | | |
| Genotypes | Hypothesis | 33914.969 | 24 | 1413.124 | 1.343 | 0.149 |
| | Error | 141018.533 | 134 | 1052.377 | | |

## Pathogenicity, infectivity and mortality in seedlings inoculated with *H. annosum* genotypes

Overall, *Heterobasidion* genotypes had similar pathogenicity, i.e. colonized similar stem lengths, on both *P. pinea* and *P. taeda*, independent of genotype origin (Fig. 2). One genotype from North America (code n. 5) caused significantly longer lesions than the others on both plant hosts (*P. pinea* $F = 4.39$, $P = 0.0003$; *P. taeda* $F = 5.07$, $P < 0.0001$). With regards to infection success across hosts, the same North American genotype showed the most seedlings infected on both pine species (Table 3). Rank correlation analysis indicated a correlation in proportion of infected seedlings (Spearman's $\rho = 0.71$, $P = 0.071$), but an insignificant correlation in stem length colonized (Spearman's $\rho = 0.36$, $P = 0.43$), when comparing results between hosts. Cumulative mortality on inoculated pines was relatively low, with 10 dead *P. pinea* and 69 dead *P. taeda* seedlings after 8 months. Logistic regression showed no differences in mortality by isolate on *P. pinea* seedlings, but for *P. taeda* seedlings, North American isolates n. 1 and 5 and European n. 16 had significantly higher mortality than that due to North American isolate code n. 7 at $P = 0.05$. No negative controls died or displayed *Heterobasidion* conidiophores.

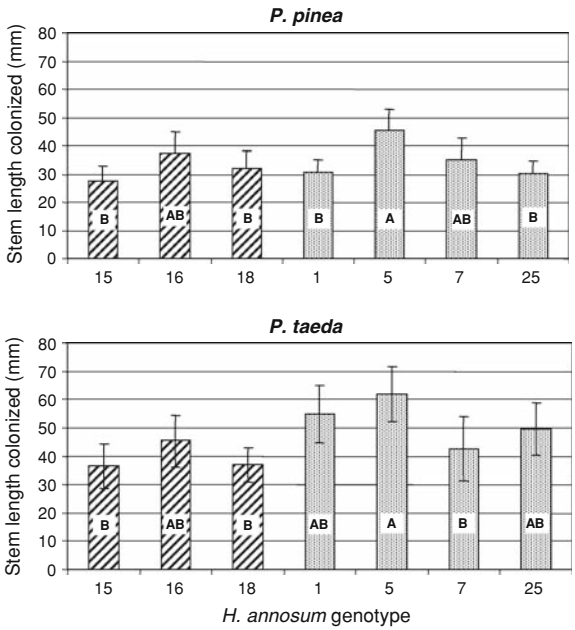


**Fig. 2** Results of comparative inoculation on European (*Pinus pinea*) and North American (*P. taeda*) host pines. *Bars* show mean stem length colonized by Eurasian-type *Heterobasidion annosum s.s.* (▨) and North American-type *H. annosum* P ISG (▩). *Error bars* show 95% confidence intervals. Genotypes not connected by the same letter are significantly different at $P = 0.05$ after post hoc Tukey HSD Test. Note that statistical analyses were conducted on $\text{Log}_{10}(x)$-transformed data. See Table 1 for genotype codes

## Inoculum pressure of the two *Heterobasidion* spp. present in the study site

A total of 562 *Heterobasidion* single-spore colonies were counted, 228 were isolated and characterized by PCR amplification. Of these, 134 (59%) were typed as *H. annosum* P ISG and 94 (41%) as *H. annosum s.s.*

Spores of *H. annosum* P ISG were collected in all four sampling periods, while spores of *H. annosum s.s.* were absent in September 2006 (Fig. 3). IF ranged from 44 to 63% and from 0 to 88% for *H. annosum* P ISG and *H. annosum s.s.*, respectively. DR of *H. annosum* P ISG ranged from 14 to 76 spores $m^2\ h^{-1}$, while DR of *H. annosum s.s.* was between 0 to 38 spores $m^2\ h^{-1}$.

Seasonal variation in propagule pressure, both in terms of IF and DR, was significant for the Eurasian

**Table 3** Logistic regression results by pine species for infectivity and mortality

| | Parameters[a] | Coefficient estimate | SE | *z* value | Pr(> \|z\|) |
|---|---|---|---|---|---|
| Infectivity: Dependent variable is infected (1) vs. not infected (0) | | | | | |
| *P. pinea* | Intercept | 1.0986 | 0.2981 | 3.6850 | 0.0002 |
| | Genotype n.1 | 0.9258 | 0.5006 | 1.8490 | 0.0644 |
| | Genotype n.15 | 0.4490 | 0.4584 | 0.9790 | 0.3274 |
| | Genotype n.16 | 0.6360 | 0.4686 | 1.3570 | 0.1747 |
| | Genotype n.5 | 2.2513 | 0.7787 | 2.8910 | 0.0038 |
| | Genotype n.25 | 1.2809 | 0.5544 | 2.3100 | 0.0209 |
| | Genotype n.18 | 0.7732 | 0.4828 | 1.6010 | 0.1093 |
| *P. taeda* | Intercept | 1.2637 | 0.3141 | 4.0230 | <0.0001 |
| | Genotype n.1 | 0.7607 | 0.5103 | 1.4910 | 0.1360 |
| | Genotype n.15 | 1.1342 | 0.5629 | 2.0150 | 0.0439 |
| | Genotype n.16 | 1.3203 | 0.6062 | 2.1780 | 0.0294 |
| | Genotype n.5 | 2.7968 | 1.0562 | 2.6480 | 0.0081 |
| | Genotype n.25 | 2.0862 | 0.7850 | 2.6580 | 0.0079 |
| | Genotype n.18 | 2.1036 | 0.7848 | 2.6800 | 0.0074 |
| Mortality: Dependent variable is survived (1) vs. died (0) | | | | | |
| *P. pinea* | Intercept | 4.0780 | 1.0080 | 4.0440 | 0.0001 |
| | Genotype n.1 | −0.7102 | 1.2380 | −0.5730 | 0.5660 |
| | Genotype n.15 | −0.0522 | 1.4260 | −0.0370 | 0.9710 |
| | Genotype n.16 | −0.7102 | 1.2380 | −0.5730 | 0.5660 |
| | Genotype n.5 | −0.0171 | 1.4260 | −0.0120 | 0.9900 |
| | Genotype n.25 | −0.0171 | 1.4260 | −0.0120 | 0.9900 |
| | Genotype n.18 | 0.0000 | 1.4260 | 0.0000 | 1.0000 |
| *P. taeda* | Intercept | 2.9267 | 0.5926 | 4.9390 | <0.0001 |
| | Genotype n.1 | −1.9151 | 0.6606 | −2.8990 | 0.0037 |
| | Genotype n.15 | −0.5288 | 0.7545 | −0.7010 | 0.4834 |
| | Genotype n.16 | −1.3792 | 0.6873 | −2.0070 | 0.0448 |
| | Genotype n.5 | −2.1035 | 0.6566 | −3.2040 | 0.0014 |
| | Genotype n.25 | −0.9214 | 0.7164 | −1.2860 | 0.1984 |
| | Genotype n.18 | −0.9024 | 0.7162 | −1.2600 | 0.2077 |

[a] Reference category is Genotype n.7 for all logistic regression analyses

*H. annosum s.s.* but it was not for the North American *H. annosum* P ISG (Table 4). This was true comparing both data from the four collection periods and data from winter samplings versus summer samplings.

While propagule pressure of the two OTUs was similar and statistically indistinguishable in winter samplings (IF, Pearson $\chi^2 = 0.6110$, d.f. = 1, $P = 0.4345$, Fisher's Exact test $P = 0.6029$; DR, Pearson $\chi^2 = 0.0130$, d.f. = 1, $P = 0.9096$, Fisher's Exact test $P = 1.0000$), propagule pressure of *H. annosum* P ISG was significantly higher than that of *H. annosum s.s.* in summer samplings (IF, Pearson $\chi^2 = 13.0670$, d.f. = 1, $P = 0.0003$, Fisher's Exact test $P = 0.0006$; DR, Pearson $\chi^2 = 46.2860$, d.f. = 1, $P < 0.0001$, Fisher's Exact test $P < 0.0001$). In Castelfusano (data not shown), the North American pathogen had comparable inoculum pressure across all samplings; the Eurasian taxon was not detected in the sampling points selected for the experiment at this site.

As expected for a region characterized by Mediterranean climate, the three meteorological parameters measured diverged significantly between the winter and summer periods. Average winter values for mean temperature, mean precipitation, and mean aridity Index were 14.1°C, 3.1 mm day$^{-1}$, and 3.9, respectively. Conversely, average summer values for

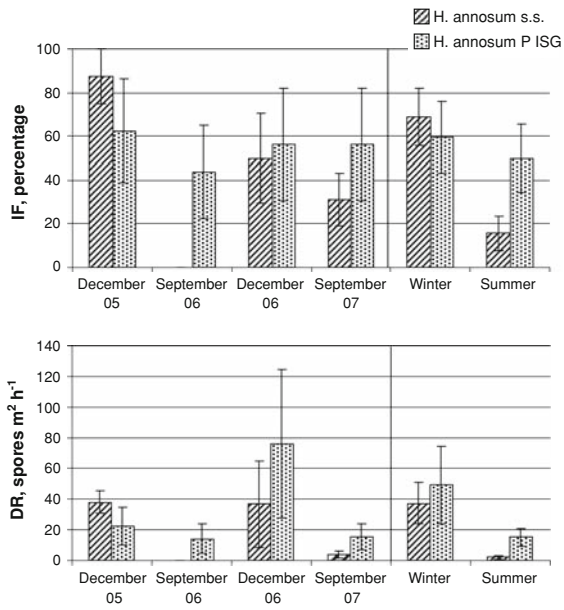


**Fig. 3** Propagule pressure of *Heterobasidion annosum sensu stricto* (*s.s.*) and *H. annosum* P ISG at four time points, from December 2005 to September 2007, in the Circeo National Park forest. Infection Frequencies (IF) represent the mean percentage of traps infected by spores of the fungus and Deposition Rates (DR) are expressed as the mean number of spores per square meters per hour. *Bars* show standard errors (SE). Winter and summer indicate the results of the two samplings performed in the same season combined

the same three parameters were 23.5°C, 1.6 mm day$^{-1}$, and 1.6. In summary, on average, winters were significantly colder, wetter, and less arid than summers.

## Discussion

Two of the experiments presented above were designed to determine whether the success of the North American *H. annosum* P ISG pathogen in Italy may be driven by increased pathogenicity or infectivity of the invasive pathogen. We did not find evidence that either parameter was increased for the invasive pathogen on the invaded host relative to the native pathogen with which the host co-evolved. However, a third experiment provided evidence that the exotic taxon is characterized by increased propagule pressure in the dry summer season with respect to the native one. Given the extended dry conditions of the areas invaded, we suggest this factor may be extremely relevant in facilitating the spread of the exotic organism.

The first experiment was designed to test pathogenicity of a significant sample of strains from the Eurasian, native North American, and invading North American pathogen populations by measuring the size of lesions caused upon inoculation of the selected set of genotypes on a broadly distributed European pine host, *P. sylvestris*. Lesion size is traditionally associated with pathogenicity as indicated by a vast body of literature (Werner and Łakomy 2002a; Bodles et al. 2007; Garbelotto et al. 2007). The three groups tested were comparable in range of pathogenicity, with the invasive strains causing the narrowest range of lesion size. Because

**Table 4** Statistical analysis for the assessment of temporal variation of propagule pressure of the two *Heterobasidion* species in the Circeo National Park forest

| | | Kruskal–Wallis test | | | $\chi^2$ test | | |
|---|---|---|---|---|---|---|---|
| | | *H* | *df*/N | *P* | $\chi^2$ | *df* | *P* |
| Comparison of data from the four collection periods between December 2005 and September 2007 | | | | | | | |
| *H. annosum* P ISG | IF[a] | 0.3140 | 3/16 | 0.9574 | 0.5429 | 3 | 0.9094 |
| | DR[b] | 0.7500 | 3/16 | 0.8614 | | | |
| *H. annosum s.s.* | IF | 9.8598 | 3/16 | 0.0198 | 15.2222 | 3 | 0.0016 |
| | DR | 10.2849 | 3/16 | 0.0163 | | | |
| Comparison of data from winter (December) samplings and summer (September) samplings | | | | | | | |
| *H. annosum* P ISG | IF | 0.1509 | 1/16 | 0.6977 | 0.2571 | 1 | 0.6121 |
| | DR | 0.5485 | 1/16 | 0.4589 | | | |
| *H. annosum s.s.* | IF | 6.7348 | 1/16 | 0.0095 | 10.7037 | 1 | 0.0011 |
| | DR | 8.1657 | 1/16 | 0.0043 | | | |

[a] IF (Infection Frequency) = percentage of infected wood traps

[b] DR (Deposition Rates) = number of viable spores per square meter per hour

genotypes were chosen haphazardly, without any previous knowledge of their pathogenicity level, we believe that variation within each group is better described by comparing the best-performing with the worst-performing isolates within each group, rather than performing multiple range tests. When variation within group was analyzed in this fashion, significant differences were found both for the Eurasian and the native North American groups, while no such differences were detected within the invasive North American group. The observed reduction in phenotypic variability may stem from a reduction in genetic variability upon the introduction of the North American taxon into Europe. Subsequent to the selection of isolates for these experiments D'Amico et al. (2007) detected a low number of mating alleles in the invasive population, and AFLP and microsatellite analyses of all three pathogen populations (M. Garbelotto et al. unpublished data) showed reduced genetic variability in the invasive population.

In the second experiment, common-garden inoculation studies onto seedlings of the frequent eastern North American host, *P. taeda*, and of the Italian *P. pinea* failed to identify a significant increased infectivity or pathogenicity of any North American genotypes on the European pine species. It should be noted that there are numerous examples of plant pathogens for which pathogenicity is successfully tested on seedlings, independently of whether the pathogen generally affects the roots, the stem or the branches of its hosts in the field (Green and MacAskill 2007; Balci et al. 2008; Thoungchaleun et al. 2008). Previous work on *H. annosum* itself confirms the validity of using seedlings to test pathogenicity levels of fungal genotypes for this pathosystem (Worrall et al. 1983; Olson and Stenlid 2001; Werner and Łakomy 2002a, b; Garbelotto et al. 2007).

Increased infectivity or pathogenicity as the result of lack of co-evolution between a plant host and an exotic pathogen would have resulted either in higher pathogenicity of all North American genotypes on the European pine host or in incongruent patterns of pathogenicity for North American isolates on the European plant host, e.g. North American genotypes characterized by low pathogenicity on the North American pine host may have displayed high pathogenicity on the European host. We did not observe either pattern. Nor did the pathogens appear to be better adapted to sympatric rather than allopatric hosts, as it has been shown for other host-pathogen systems (Ebert and Bull 2008). Instead, North American and European strains showed extremely congruent results when lesion size and percentage of infected seedlings were compared between the two plant hosts studied. These results are in agreement with those presented by Bonello et al. (2008) who found that when one invasive North American and one native Eurasian genotype were inoculated on Italian stone pine seedlings, lesions caused by the invasive were either smaller than or indistinguishably sized from those caused by the native over two trials. The native North American genotype n. 5, caused significantly larger lesions and colonized a higher proportion of seedlings, but it did so both in *P. pinea* and *P. taeda* and not only in the evolutionarily novel North American pathogen/Italian host combination. Although n. 5 had low pathogenicity on *P. sylvestris* in Italian inoculations, its anomalous growth after re-isolation suggests alteration by acquisition of a virus or other external factor during this experiment (see Methods) and not a pathogen-by-host interaction in relative pathogenicity.

We commenced studying this invasion approximately 60 years after the likely introduction of North American *H. annosum* P ISG to Italy, so the pathogen currently present in the field has reproduced for many generations under strong selection and may differ in key ways from the founders. For this reason, we included genotypes both from the presumed source population for the invasion, the south eastern US, and from the invasion area in order to detect possible invasion-associated factors that may have lead to a differentiation of invasive genotypes (Hufbauer and Torchin 2007), but our tests failed to detect any significant differences between source and invasive genotypes. Nonetheless, successful invasion of an exotic species in a new environment is likely to be facilitated by multi-factorial and interacting causes. Although the lack of co-evolution between host and exotic pathogen is a very common hypothesis explaining invasiveness of plant pathogens, failure to find evidence for this scenario does not preclude a role for evolutionary factors in this pathosystem. Indeed, hybrid genotypes have actually been detected in the zone of invasion (Gonthier et al. 2007), a finding that indicates that this type of rapid evolution may have influenced the success of North American genotypes.

The observation that the invasive North American OTU was abundant in dry pine stands mostly uninhabited by the native taxon (see Gonthier et al. 2007) led to our experiment measuring whether the two differed in their reproductive potential in this environment. This is a crucial variable, as propagule pressure has been identified as one of the important ecological traits characterizing successful invasive species (Williamson and Fitter 1996; Sakai et al. 2001; Hawkes 2007) and reproductive rate underlies the threshold for epidemic disease emergence (Woolhouse et al. 2005). Furthermore, the ability of a pathogen to reproduce in dry conditions is important in this case, because the overwhelming majority of Mediterranean forest ecosystems are characteristically dry most of the year. Although evidence directly linking disease severity with inoculum potential is lacking for forest root pathogens whose spread may be mostly caused by secondary growth of fungal vegetative structures (e.g. mycelium or rhizomorphs) from tree to tree (Garbelotto 2004), in the case of *Heterobasidion*, all evidence shows that primary infections caused by airborne basidiospores are essential in starting new infections, and thus are directly responsible for the rate of infestation of a forest (Rishbeth 1951; Garbelotto et al. 1999).

Spores of this pathogen are produced by perennial fruitbodies. As such, its reproductive potential, although variable through time, is less susceptible to daily fluctuations than that of fungi whose spores are produced by ephemeral and short-lived fruitbodies. James and Cobb (1984) reported that spores of *Heterobasidion* were present all year-round in California forests, and in a study of fungal airspora in southern Spain, *Ganoderma*, a species that like *Heterobasidion* produces perennial shelf-like fruitbodies, displayed less pronounced seasonal variation in spore production than other annual fungi (Morales et al. 2006). Thanks to this property, spore production patterns for a period of several weeks can be reliably studied with a 24 h-long trapping.

Results from basidiospore trapping in the third experiment clearly indicated that propagule pressure of the North American invasive taxon was not affected by season, during the course of our experiment. The seasonal consistency of spore deposition by invasive genotypes is further supported by the results of a similar experiment, conducted in the same period, at Castelfusano where only the North American taxon is present in significant amounts (data not shown). By contrast, the reproductive potential of the native *H. annosum* was significantly lower in the dry summer samplings than in the rainy winter ones.

To further compound the significance of the difference measured, while North American genotypes remain above the threshold of airspora needed for successful tree or stump infection, i.e. 10 spores $m^{-2}\ h^{-1}$ (Moykkynen and Kontiokari 2000), the native European ones clearly fall below such threshold during the summer time. These findings are congruent with observational data that the differential success of introduced fungi may be related to life-history traits conducive to spore transmission (Schwartz et al. 2006). Overall, DR values observed during our experiment were relatively lower than those reported in the literature for the complex *H. annosum* (reviewed in Redfern and Stenlid 1998), but are consistent with data from previous surveys in central Italy (Gonthier et al. 2007). In Europe, levels of inoculum comparable to those found in this study were previously reported for *H. annosum sensu stricto*, which displayed DR values significantly lower than the other *Heterobasidion* species (Gonthier et al. 2005).

Whether the consistent spore release of the invasive species may be an adaptive phenotypic change that evolved in the North American taxon after introduction, or whether it may have been a trait present in the founding population, remains unknown at present. Studies of the North American P ISG type in the south eastern US show reduction in sporulation and stump infection during the hot summer months (Boyce 1963, 1966; Driver and Ginns 1964), but in a western North American region characterized by Mediterranean conditions, *H. annosum* P ISG shows year-round sporulation (James and Cobb 1984).

Several environmental factors may influence the consistent sporulation of the invasive *H. annosum* compared with the seasonal sporulation of native genotypes. Temperature has been identified as a potential driver of temporal sporulation and infection dynamics (Driver and Ginns 1964; Gonthier et al. 2005), and seasonal differences in moisture may also be important. An analysis of metereological data indicate that our September samplings were performed at the end of a dry and warm period, significantly different from the much wetter and

colder period preceding our December samplings. Interestingly, the two Mediterranean areas known for a significant presence of the native Eurasian species are the Forest of Mesola outside the zone of infestation in northern Italy (M. Garbelotto and P. Gonthier unpublished data), and the Circeo National Park forest at the southern edge of the zone of infestation by the North American taxon (Gonthier et al. 2007). Both areas are anomalous ecologically: the first, being at the very edge of the range of *P. pinea*, is characterized by relatively high levels of precipitation and by a much shorter dry season, the second displays a flora characteristically non-Mediterranean due to its existence on reclaimed swampy terrain characterized by extreme abundance of underground water. In North America, the genetically heterogeneous *H. annosum* P ISG causes widespread disease in both mesic and xeric environments (Filip and Morrison 1998).

The extensive mortality of *P. pinea* trees associated with the invasive *H. annosum* P ISG in the dry coastal pine stands around Rome is unprecedented and represents an example of an emergent fungal disease with significant ecological consequences for the affected habitats. Relatedness between the major natural host pines and the fact that the native and invasive pathogenic taxa are the closest relatives within the *Heterobasidion* species complex provide a unique fungal system to analyze the importance of different factors that may be promoting invasiveness. The ability to disentangle specifically which factors are contributing to *Heterobasidion* disease emergence provides valuable data for potential control mechanisms, for limiting future introductions of similar exotic microbes, and for broadening our understanding of biotic invasions of introduced fungi. We did not find evidence that this emergent disease is caused by a disproportionate pathogenicity and infectivity levels of the exotic causal agent on a naïve host. However, we did find that differences in propagule pressure during the summer may be an important driver of disease severity in the dry Italian coastal forest environment. Until the arrival of the exotic species, these trees may have rarely been challenged by the native microbe, because its presence in this niche is minimal, even outside the invaded zone (Gonthier et al. 2007). This successful invasion, however, is likely to be attributed to multiple interacting ecological and evolutionary processes and events: ongoing research will continue to elucidate the temporal sequence and relative importance of both.

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## References

Asiegbu FO, Adomas A, Stenlid J (2005) Conifer root and butt rot caused by *Heterobasidion annosum* (Fr.) Bref. *s.l.* Mol Plant Pathol 6:395–409

Baker HG (1965) Characteristics and modes of origins of weeds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, London, pp 141–172

Balci Y, Balci S, MacDonald WL, Gottschalk KW (2008) Relative susceptibility of oaks to seven species of *Phytophthora* isolated from oak forest soils. For Pathol 38: 397–409

Blumenthal DM (2006) Interactions between resource availability and enemy release in plant invasion. Ecol Lett 9:887–895

Bodles WJA, Beckett E, Woodward S (2007) Responses of Sitka spruce of different genetic origins to inoculation with *Heterobasidion annosum*: lesion lengths, fungal growth and development of the lignosuberized boundary zone. For Pathol 37:174–186

Bonello P, Capretti P, Luchi N, Martini V, Michelozzi M (2008) Systemic effects of *Heterobasidion annosum s.s.* infection on severity of *Diplodia pinea* tip blight and terpenoid metabolism in Italian stone pine (*Pinus pinea*). Tree Physiol 28:1653–1660

Boyce JS Jr (1963) Colonization of pine stem sections by *Fomes annosus* and other fungi in two slash pine stands. Plant Dis Report 47:320–324

Boyce JS Jr (1966) Sporulation by *Peniophora gigantea* with reference to control of Annosus root rot. For Sci 12:2–7

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7:721–733

Čufar K, De Luis M, Eckstein D, Kajfež-Bogata L (2008) Reconstructing dry and wet summers in SE Slovenia from oak tree-ring series. Int J Biometeorol 52:607–615

D'Amico L, Motta E, Annesi T, Scirè M, Luchi N, Hantula J, Korhonen K, Capretti P (2007) The North American P group of *Heterobasidion annosum* s.l. is widely distributed in *Pinus pinea* forests of the western coast of central Italy. For Pathol 37:303–320

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. J Ecol 88:528–534

De Martonne E (1926) Une nouvelle fonction climatologique: l'indice d'aridité. La Metéorologie 2:449–458

Despommier D, Ellis BR, Wilcox BA (2007) The role of ecotones in emerging infectious diseases. EcoHealth 3:281–289

Desprez-Loustau ML, Robin C, Buee M, Courtecuisse R, Garbaye J, Suffert F, Sache I, Rizzo DM (2007) The fungal dimension of biological invasions. Trends Ecol Evol 22:472–480

Dodd RS, Hüberli D, Douhovnikoff V, Harnik TY, Afzal-Rafii Z, Garbelotto M (2004) Is variation in susceptibility to *Phytophthora ramorum* correlated with population genetic structure in coast live oak (*Quercus agrifolia*)? New Phytol 165:203–214

Driver CH, Ginns JH Jr (1964) The effects of climate on occurrence of Annosus root-rot in thinned slash pine plantations. Plant Dis Report 48:509–511

Duncan RP, Blackburn TM, Sol D (2003) The ecology of bird introductions. Annu Rev Ecol Syst 34:71–98

Ebert D, Bull JJ (2008) The evolution and expression of virulence. In: Stearns SC, Koella JC (eds) Evolution in health and disease, 2nd edn. Oxford University Press, Oxford, pp 153–167

Elton CS (1958) The ecology of invasions by animals and plants. Methuen, London

Facon B, Pointier JP, Glaubrecht M, Poux C, Jarne P, David P (2003) A molecular phylogeography approach to biological invasions of the New World by parthenogenetic Thiarid snails. Mol Ecol 12:3027–3039

Facon B, Genton BJ, Shykoff JA, Jarne P, Estoup A, David P (2006) A general eco-evolutionary framework for understanding bioinvasions. Trends Ecol Evol 21:130–135

Fausch KD (2008) A paradox of trout invasions in North America. Biol Invasions 10:685–701

Filip GM, Morrison DJ (1998) North America. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann R (eds) *Heterobasidion annosum*, biology, ecology, impact and control. CAB International, New York, pp 405–427

Fitt DBL, McCartney HA, Walkalate PJ (1989) The role of rain in dispersal of pathogen inoculum. Annu Rev Phytopathol 27:241–270

Garbelotto M (2004) Root and butt rot diseases. In: Burley J, Evans J, Youngquist JA (eds) The encyclopedia of forest sciences, vol 2. Elsevier, Oxford, pp 750–758

Garbelotto M, Schmidt D (2009) Phosphonate controls sudden oak death pathogen for up to 2 years. Calif Agric 63:10–17

Garbelotto M, Cobb FW, Bruns TD, Otrosina WJ, Popenuck T, Slaughter G (1999) Genetic structure of *Heterobasidion annosum* in white fir mortality centers in California. Phytopathology 89:546–554

Garbelotto M, Gonthier P, Nicolotti G (2007) Ecological constraints limit the fitness of fungal hybrids in the *Heterobasidion annosum* species complex. Appl Environ Microbiol 73:6106–6111

Gernandt DS, Lopez GG, Garcia SO, Liston A (2005) Phylogeny and classification of *Pinus*. Taxon 54:29–42

Gilbert GS (2002) Evolutionary ecology of plant diseases in natural ecosystems. Annu Rev Phytopathol 40:13–43

Gonthier P, Warner R, Nicolotti G, Mazzaglia A, Garbelotto MM (2004) Pathogen introduction as a collateral effect of military activity. Mycol Res 108:468–470

Gonthier P, Garbelotto M, Nicolotti G (2005) Seasonal patterns of spore deposition of *Heterobasidion* species in four forests of the western Alps. Phytopathology 95:759–767

Gonthier P, Nicolotti G, Linzer R, Guglielmo F, Garbelotto M (2007) Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. Mol Ecol 16:1389–1400

Green S, MacAskill GA (2007) Pathogenicity of *Marssonina betulae* and other fungi on birch. Plant Pathol 56:242–250

Hawkes CV (2007) Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. Am Nat 170:832–843

Hufbauer RA, Torchin ME (2007) Integrating ecological and evolutionary theory of biological invasions. In: Nentwig W (ed) Biological invasions. Springer, Berlin, pp 79–96

James RL, Cobb FW Jr (1984) Spore deposition by *Heterobasidion annosum* in forests of California. Plant Dis 68:246–248

Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol Lett 8:558–574

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Korhonen K, Stenlid J (1998) Biology of *Heterobasidion annosum*. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann R (eds) *Heterobasidion annosum,* biology, ecology, impact and control. CAB International, New York, pp 43–70

Korhonen K, Capretti P, Karjalainen R, Stenlid J (1998) Distribution of *Heterobasidion annosum* intersterility groups in Europe. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann R (eds) *Heterobasidion annosum* biology, ecology, impact and control. CAB International, New York, pp 93–104

Lambrinos JG (2004) How interactions between ecology and evolution influence contemporary invasion dynamics. Ecology 85:2061–2070

Linzer RE, Otrosina W, Gonthier P, Bruhn J, Laflamme G, Bussières G, Garbelotto M (2008) Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and of human-mediated, long-range dispersal. Mol Phylogenet Evol 46:844–862

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710

Malmstrom CM, McCullough AJ, Johnson HA, Newton LA, Borer ET (2005) Invasive annual grasses indirectly increase virus incidence in California native perennial bunchgrasses. Oecologia 145:153–164

Mayr E (1965) The nature of colonizations in birds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, London, pp 29–43

Morales J, Gonzáles-Minero FJ, Carrasco M, Ogalla VM, Candau P (2006) Airborne basidiospores in the atmosphere of Seville. Aerobiologia 22:127–134

Moykkynen T, Kontiokari J (2000) Spore deposition of *Heterobasidion annosum* coll. in *Picea abies* stands of North Karelia, eastern Finland. For Pathol 31:107–114

Olson A, Stenlid J (2001) Mitochondrial control of fungal hybrid virulence. Nature 411:438

Parker IM, Gilbert GS (2004) The evolutionary ecology of novel plant-pathogen interactions. Annu Rev Ecol Syst 35:675–700

Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv Biol 17:59–72

Prinzing A, Durka W, Klotz S, Brandl R (2002) Which species become alien? Evol Ecol 4:385–405

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Redfern DB, Stenlid J (1998) Spore dispersal and infection. In: Woodward S, Stenlid J, Karjalainen R, Hüttermann R (eds) *Heterobasidion annosum,* biology, ecology, impact and control. CAB International, New York, pp 105–124

Rishbeth J (1951) Observations on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations. (III) Natural and experimental infection of pines, and some factors affecting severity of the disease. Ann Bot 15:221–246

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neill P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Sax DF, Brown JH (2000) The paradox of invasion. Glob Ecol Biogeogr 9:363–371

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecol Lett 9:501–515

Scirè M, D'Amico L, Motta E, Annesi T (2008) North American P type of *Heterobasidion annosum* shows pathogenicity towards *Pinus halepensis* in Italy. For Pathol 38:299–301

Swedjemark G, Karlsson B (2006) Mycelial growth and exclusion of *Heterobasidion parviporum* inoculated in branches of 15-year-old *Picea abies* clones. For Pathol 36:209–214

Tatem AJ, Rogers DJ, Hay SI (2006) Global transport networks and infectious disease spread. Adv Parasitol 62:293–343

Thoungchaleun V, Kim KW, Lee DK, Kim CS, Park EW (2008) Pre-infection behavior of the pitch canker fungus *Fusarium circinatum* on pine stems. Plant Pathol J 24:112–117

Togashi D, Shigesada N (2006) Spread of the pinewood nematode vectored by the Japanese pine sawyer: modeling and analytical approaches. Pop Ecol 48:271–283

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421:628–630

Werner A, Łakomy P (2002a) Host specialization of IS-group isolates of *Heterobasidion annosum* to Scots pine, Norway spruce and common fir in field inoculation experiments. Dendrobiology 47:59–68

Werner A, Łakomy P (2002b) Intraspecific variation in *Heterobasidion annosum* for mortality rate on *Pinus sylvestris* and *Picea abies* seedlings growth in pure culture. Mycologia 94:856–861

Williamson MH, Fitter A (1996) The characters of successful invaders. Biol Conserv 78:163–170

Wingfield MJ, Slippers B, Roux J, Wingfield BD (2001) Worldwide movement of exotic forest fungi, especially in the tropics and the southern hemisphere. Bioscience 51:134–140

Woodward S, Stenlid J, Karjalainen R, Hüttermann A (eds) (1998) *Heterobasidion annosum*: biology, ecology, impact and control. CAB International, Wallingford

Woolhouse MEJ, Haydon DT, Antia R (2005) Emerging pathogens: the epidemiology and evolution of species jumps. Trends Ecol Evol 20:238–244

Worrall JJ, Parmeter JR, Cobb FW (1983) Host specialization of *Heterobasidion annosum*. Phytopathology 73:304–307