The alien invasive forest pathogen *Heterobasidion irregulare* is replacing the native *Heterobasidion annosum*

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**Abstract** Invasions by alien pathogens are a major threat to forest conservation. The North American fungal pathogen of conifers *Heterobasidion irregulare*, inadvertently introduced in Central Italy in the 1940s, has been spreading causing high mortality of Italian stone pine (*Pinus pinea*). While invading new-found niches, *H. irregulare* has established itself in the current range of the native congener *H. annosum*. The aims of this study were to determine whether in time: (I) *H. irregulare* populations may be increasing in size; (II) *H. irregulare* may be replacing *H. annosum*, rather than simply coexisting with it; and, (III) *H. annosum* may disappear in forests infested by *H. irregulare*. The presence, abundance and distribution of *H. annosum* and *H. irregulare* were assessed through an aerobiological assay replicated ten years apart in a forest in which both species have been coexisting. Replacement index (RI), Markov chains and geometric progressions were used to model the interspecific interaction between the two species and to assess the invasiveness of *H. irregulare*. Results showed that, in 10 years, the incidence of *H. annosum* dropped from 39.4 to 6.1%, while that of *H. irregulare* increased from 57.6 to 81.8%, with the alien pathogen replacing the native species (RI = 84.6%) and spreading at a maximum rate of 139 ha/year. Although our models show that the extinction of *H. annosum* may be unlikely, the ability of *H. irregulare* to replace it suggests the alien pathogen may also readily colonize those parts of Europe where *H. annosum* is more abundant than in Central Italy.

**Keywords** Biological invasion · Fungi · Modelling · Pine · Plant pathogens · Spores

**Introduction**

The threat posed by alien forest pathogens to naive forests is well recognized (Lovett et al. 2016). Once introduced and established, alien pathogens may become invasive by spreading at a rate that is affected by many factors, including, but not limited to, their reproductive potential, their ability to withstand disturbances, competition and/or predation, their dispersal ability, the density of susceptible and/or of infectious hosts, environmental and climatic factors,
and the permeability of the landscape (Garbelotto and Pautasso 2011). A frequent invasion scenario involves alien organisms exploiting niches unoccupied by native organisms. With regards to plant pathogenic fungi, this is the case, for instance, of the invasion by the Asian chestnut blight fungal pathogen *Cryphonectria parasitica* in both North America and Europe. In North America, where the native American chestnut (*Castanea dentata*) is highly susceptible, its spread rate has been estimated at more than 30 km per year, resulting in devastating effects throughout its native distribution range in a few decades (Evans and Finkral 2010; Rigling and Prospero 2017). However, introduced pathogens and pests frequently interact with pre-existing organisms, both native or themselves introduced. One of the most notable examples includes the causal agents of Dutch elm disease *Ophiostoma novo-ulmi* introduced where the related *O. ulmi* was already well established (Brasier and Buck 2001). The fitness advantage exhibited by *O. novo-ulmi* compared to *O. ulmi* led to a very successful invasion of the former species and to a replacement of the less fit *O. ulmi* by *O. novo-ulmi*, possibly due to direct competitive antagonism between the two (Brasier and Buck 2001).

While it is acknowledged that asymmetry in fitness between two species with ecological overlap is responsible for the dominance of one over the other, the mechanisms underlying the dominance and maybe even the replacement of one species by another are multiple (see Reitz and Trumble 2002; Short and Petren 2011) and involve direct (e.g. antagonism) and indirect (e.g. better resource utilization) interactions, as well as demographic (e.g. reproductive and transmission potentials) and metapopulation level dynamics (e.g. migration rates of each species). It should be noted that not all interactions need to be antagonistic: for instance, the alien organism here studied, *Heterobasidion irregulare*, has been reported to increase its sporulation potential when sympatric with the native congeneric *H. annosum* (Giordano et al. 2019). Likewise, priority effects may favor less fit pathogens which arrive first on a substrate or a host (Hood 2003; Simpson et al. 2003; Al-Naimi et al. 2005; Laine 2011). Additionally, interfertility between interacting species further complicates the scenarios by allowing individuals belonging to two species to coexist in the same physical space where they can mate and exchange advantageous adaptive genes or deleterious infectious agents (Paoletti et al. 2005; Hessenauer et al. 2020; Sillo et al. 2021).

The role played by each factor in determining the outcome of a biological invasion may be hard to assess when two or more species interact with one another. Normally, such role has been inferred based on data that simply document changes in population size of each interacting species in any given area. Unfortunately, those data often have limited geographic detail and/or are obtained from different and differently designed studies (Short and Petren 2011; Downey and Richardson 2016). Repeat surveys over time across a predetermined set of sampling points may be one of the best ways to obtain a better understanding of whether alien invasive species may be: I) increasing in populations size or carrying capacity, II) increasing the proportion of their overall representation, III) broadening their geographic range and, IV) replacing native individuals. In a recent publication, Kozanitas et al. (2017) through a repeated sampling of individual trees across multiple transects and sites were able to show the alien pathogen *Phytophthora ramorum* was directly replacing the native pathogen *Phytophthora nemorosa*, at rates that increased with increasing rainfall. A similar study showed a similar replacement on Ash of the native and largely saprobic fungus *Hymenoscyphus albidus* by the alien and pathogenic *Hymenoscyphus fraxineus* (Mckinney et al. 2012).

Studies employing repeated samplings and spatially defined sampling schemes may also provide information useful to both predict the rate of future spread of the invasive organism and to reconstruct its past spread history. Although this information is necessarily site specific, it may provide invaluable baseline information that combined with other information such as the introduction date of the exotic pathogen, the original introduction location and data on landscape fragmentation at various geographic scales, may help refine our understanding of the expansion potential of the alien organism. This type of information is of pivotal importance to predict the outcomes of invasions and to make decisions on eradication or containment measures, but, unfortunately, it is often not readily available for introduced forest pathogens.

A relatively recently discovered biological invasion is that of the North American fungal plant pathogen *H. irregulare*, inadvertently introduced a few
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in terms of deposition rates of airborne propagules (i.e. basidiospores) was comparable between the two Heterobasidion species (Gonthier et al. 2012). Therefore, the Sabaudia forest appears ideal not only to study the invasiveness of H. irregulare, but also to test whether this fungus may or may not be replacing the native H. annosum.

In this study, we compared the results of two samplings performed in the same points across the Sabaudia forest ten years apart, the first in 2006 and the second in 2016. Samplings and analyses followed an identical methodology and were aimed at answering the following questions: I) Did the proportional representation of the alien species in the Sabaudia forest overall increase between 2006 and 2016? II) Was the abundance of the alien pathogen in the Sabaudia forest overall larger in 2016 than in 2006? III) In 2016, was the exotic species present where it was absent in 2006? IV) In how many of the resampled points, had the native pathogen been replaced by the alien one and vice versa, and was the overall replacement rate symmetrical or asymmetrical? Finally, the approach used in our study, by detailing the changes in pathogen status at each sampling point between 2006 and 2016, with possible status being: a) “no pathogen”, b) “both pathogens”, c) “H. annosum only”, and, d) “H. irregulare only”, allowed us to generate both a “forward” predictive model on future changes in pathogen incidence and pathogen composition and a “backward” historical reconstruction to determine the likely date of arrival of the exotic pathogen in the Sabaudia forest. That date was then used together with the introduction date of the pathogen in Italy to determine the regional spread rate of the pathogen in the presence of habitat fragmentation. Providing data corroborating the invasive nature of H. irregulare, documenting whether it is replacing the native H. annosum, and estimating its spread rate in invaded ecosystems are all essential prerequisites to better quantify the threat level this alien North American pathogen represents for Europe.

Materials and Methods

Study site

The study was conducted in the Sabaudia Forest, a 3030 ha mixed-species woodland with a dominance of Quercus and Pinus spp., located in the Circeo National Park, a protected area of the western Mediterranean coast of the Lazio Region, Italy (336,000 m E, 4,578,000 m N–WGS84/UTM – elevation 40 m a.s.l.). A detailed description of the site is reported in Gonthier et al. (2012). The Sabaudia Forest was deemed as the most suitable site to deploy our experimental design thanks to its set of rather unique conditions: I) the site is located within the invasion area of the alien fungal pathogen Heterobasidion irregulare in Central Italy (Gonthier et al. 2014); II) both the native H. annosum and the alien congener H. irregulare coexist and sporulate in the same stand, and were present a comparable incidences at the beginning of the study (Gonthier et al. 2007, 2012); and III) no anthropic disturbances such as thinnings, cuttings and fires have been reported in the area.

Spore samplings and laboratory analyses

Spore samplings at the Sabaudia Forest were conducted to assess and compare the presence, abundance and distribution of both H. annosum and H. irregulare both in 2006 and in 2016. In December 2006, 33 long-term sampling points were located across the entire forest following a random spatial pattern design. Such points were a subset of those used to investigate the ecological association between vegetation types, H. annosum and H. irregulare in Gonthier et al. (2012). The coordinates (m) of each sampling point were recorded with a GPS device (Magellan® MobileMapper – precision ±1 m) in the WGS84/UTM projection system. Both in 2006 and 2016, the spore deposition rates (DR, in spores·m⁻²·h⁻¹) of H. annosum and H. irregulare were assessed at each sampling point based on the wood disc exposure method described in Gonthier et al. (2001, 2005, 2007, 2012). The above method is currently recommended by the European and Mediterranean Plant Protection Organization (EPPO) to detect the presence and quantify the abundance of H. irregulare (see EPPO 2020a,b and Online Resource 1 – Sect. 1). To determine the proportion of DR
attributable to *H. annosum* or *H. irregulare*, up to 10 colonies per disc were isolated and identified at the species level as described in Gonthier et al. (2012) (see Online Resource 1 – Sect. 2). For each sampling point, the proportion of colonies of either fungal species (in %) was taken as a proxy to assign a specific DR to *H. annosum* and *H. irregulare* (Gonthier et al. 2012).

Statistical analyses and modelling

The spatial pattern of the sampling points located in the study site was assessed by computing and testing the Clark and Evans aggregation index (R) (Clark and Evans 1954) (Online Resource 1 – Sect. 3.1). The overall incidences of *H. annosum* and *H. irregulare* were calculated as the ratio (%) between the number of points where the fungal species were detected and the total number of sampling points. The incidence/average DR of *H. annosum* and *H. irregulare* were compared between and within 2006 and 2016 by using conditional inference tree models (ctree) (Hothorn et al. 2006; Hothorn and Zeileis 2015) as reported in Lione et al. (2020).

Points sampled in 2006 and 2016 were split among the four mutually exclusive categories below: points where only *H. annosum* was detected (coded as Ha), points that were positive only to *H. irregulare* (Hi), points where both fungal species coexisted (Ha+Hi), or points where both species were absent (Ø) (Online Resource 1 – Sect. 3.2). The frequencies of sampling points scored as either Ha, Hi, Ha+Hi, or Ø were compared within each sampling year, while the frequencies of points classified in the same category were compared between sampling years. In both cases, comparisons were carried out with a n-sample test for the equality of proportions with continuity and Bonferroni corrections (Crawley 2013).

To test if species replacement had occurred between 2006 and 2016, a replacement index was calculated for two possible scenarios: the first with *H. irregulare* replacing *H. annosum*, and the second with *H. annosum* replacing *H. irregulare*. For the first scenario, the replacement index (RI1, in %) was calculated as the ratio between the number of points where in 2016 only *H. irregulare* was detected ($\sum_{2016}(Hi)$) and the number of the same points where in 2006 *H. annosum* was present alone ($\sum_{2006}(Ha)$) or in coexistence with *H. irregulare* ($\sum_{2006}(Ha + Hi)$), namely

$$ RI_1 = \frac{\sum_{2016}(Hi)}{\sum_{2006}(Ha) + \sum_{2006}(Ha + Hi)} $$

For the second scenario, the replacement index (RI2) was calculated in a similar way as

$$ RI_2 = \frac{\sum_{2016}(Ha)}{\sum_{2006}(Hi) + \sum_{2006}(Ha + Hi)} $$

with $\sum_{2016}(Ha)$ and $\sum_{2006}(Hi)$ indicating the number of points where only *H. annosum* or *H. irregulare* were present in 2016 and 2006, respectively. The two replacement indexes were compared with an exact version of the two-sample Poisson test (Huffman 1984) verifying: I) whether $RI_1 > RI_2$ (i.e. scenario 1 was the most likely, with *H. irregulare* replacing *H. annosum*); or II) $RI_1 < RI_2$ (i.e. scenario 2 was the most likely, with *H. annosum* replacing *H. irregulare*); or finally III) $RI_1 = RI_2$ (i.e. no replacement of either fungal species is likely to have occurred between 2006 and 2016).

To predict the future trend in the incidence of *H. annosum* and *H. irregulare*, a Markov chain model was fitted based on Dobrow (2016). The Markov matrix (M) storing the transition probabilities was calculated for the four categories (i.e. states) Ha, Hi, Ha+Hi, and Ø (Online Resource 1 – Sect. 3.3). The long-term trend of the incidence was appraised through the product of the row vector v, representing the distribution of the sampling points among the four states, and the n-step Markov matrices $M^n$, with n being an integer ranging from 2 to $k + 1$. The value of k was calculated by applying the numerical approximation algorithm reported in Dobrow (2016), until the convergence condition required for a stable equilibrium was achieved (Online Resource 1 – Sect. 3.4).

To infer what the incidence of *H. irregulare* may have been in past years and assess when the alien pathogen may have first become established in the study site, a geometric progression model (Calter and Calter 2011) with equation $I(Hi)_{t-10} = c \cdot I(Hi)_t$ was fit, with $I(Hi)_t$ being the incidence of *H. irregulare* at a given time indicated by the subscript, t being an integer representing a 10-year backward step since 2016 to 1916, and c representing the common ratio of the geometric progression. The constant c was assessed from the observed data as $c = I(Hi)_{2006}/I(Hi)_{2016}$. Based on the sampling size, the detection threshold to assess the lowest incidence value of *H. irregulare* was estimated at 3% (1 out of 33 sampling points). The abscissa of the intersection between the graph of the geometric progression and the detection threshold was deemed as a proxy of the
year in which *H. irregulare* established in the study site. An overall incidence model for *H. irregulare* was generated by merging the results from the geometric progression (backward model, appraising the past incidence), the spore samplings (observed data, showing the real incidence), and the Markov chain (forward model, forecasting the future incidence), accounting for models uncertainty (Online Resource 1 – Sect. 3.5).

Based on the outcomes of the geometric progression model, updated estimates of the linear spread rate (km/year) of *H. irregulare* across the invasion area in Central Italy were calculated through the ratio between the distance invaded (i.e. distance separating the site of first introduction and the study site) and the time needed for the invasion (i.e. time-lapse between the year when the first introduction in Italy occurred and the estimated year in which *H. irregulare* established in the study site) (Online Resource 1 – Sect. 3.6). Estimates of the surface spread rate (ha/year) were assessed from the overall incidence model dividing the area of the study site invaded by *H. irregulare* since its establishment by the time-lapse between each model step (Online Resource 1 – Sect. 3.6).

Statistical analyses and modelling were conducted with R version 3.6.0 (R Core Team 2019) (Online Resource 1 – Sect. 3.7). The significance threshold was set to 0.05 for all tests, while confidence intervals were calculated as reported in Online Resource 1 – Sect. 3.8).

**Results**

The results of the Clark and Evans aggregation index (*R* = 1.180, *P* > 0.05) confirmed that our sampling pattern was spatially random. Our observations during field surveys and samplings confirmed that no significant anthropic disturbances such as thinnings, cuttings and fires had occurred in between the two samplings. Such observations were confirmed by personal interviews with the Circeo National Park staff. Spore samplings confirmed the presence of both fungal species, i.e. the native *H. annosum* and the alien invasive *H. irregulare*, in both 2006 and 2016. However, the incidence of *H. annosum* dropped from 39.4% (23.6–57.8% CI95%) to 6.1% (1.1–19.2% CI95%) between 2006 and 2016, while that of *H. irregulare* increased from 57.6% (40.1–73.2% CI95%) to 81.8% (65.5–91.8% CI95%) in the same time period. Differences among incidences were significant (*P* < 0.05) (Fig. 1). In 2006, deposition rates DR of *H. annosum* (31.0 spores·m⁻²·h⁻¹, 14.1–71.2 spores·m⁻²·h⁻¹ CI95%) and *H. irregulare* (32.4 spores·m⁻²·h⁻¹, 15.8–75.0 spores·m⁻²·h⁻¹ CI95%) were comparable (*P* > 0.05) (Fig. 2). Conversely, 10 years later, the DR of *H. annosum* (0.79 spores·m⁻²·h⁻¹, 0–2.2 spores·m⁻²·h⁻¹ CI95%) was significantly lower (*P* < 0.05) than the DR of *H. irregulare* (117.4 spores·m⁻²·h⁻¹, 69.4–194.4 spores·m⁻²·h⁻¹ CI95%) (Fig. 2).

In 2006, sampling points where only one of the two fungal species could be detected were 12.1% of the total for *H. annosum* (Ha) and 27.3% for *H. irregulare*, while the remaining 60.6% of points was equally split in points where: a) either both pathogens were present (Ha + Hi), or b) none were present (Ø) (Table 1). No significant differences (*P* > 0.05) were found when comparing the frequencies of sampling points among the four categories Ha, Hi, Ha + Hi and Ø in 2006 (Table 1). Conversely, in 2016, most of the points (75.7%) were included in the category Hi, since only spores of *H. irregulare* were detected in the corresponding spore traps. The remaining points were split with comparable frequencies (*P* > 0.05) among Ha (0%), Ha + Hi (6.1%), and Ø (18.2%), all significantly lower (*P* < 0.05) than the percentage of points occupied by Hi (Table 1). Significant changes (*P* < 0.05) were observed for Hi (+45.4% of sampling points) and for Ha + Hi (-21.2%) between 2006 and 2016, while the decrease in frequency of either Ha or Ø (-12.1% each) was not significant (*P* > 0.05) (Table 1).

The index RI₁ of the scenario envisaging the replacement of *H. annosum* by *H. irregulare* attained a value of 84.6% (CI95% = 56.6–97.2%), while the index RI₂ modelling the replacement of *H. irregulare* by *H. annosum* was significantly lower at 0% (CI95% = 0–16.9%) (*P* < 0.05) (Online Resource 2). Since RI₁ > RI₂, scenario 1 was the most likely to have occurred, with *H. irregulare* replacing *H. annosum*. The Markov matrix M showed that, between 2006 and 2016, no sampling points switched their state from Ha, Hi, Ha + Hi, or Ø to Ha. Conversely, most of the points that in 2006 were classified in either Ha, Hi, Ha + Hi, or Ø switched to the state Hi in 2016, with a transition probability...
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Fig. 1 Conditional inference tree model comparing the incidences of *Heterobasidion annosum* and *H. irregulare* between 2006 and 2016. Barcharts reported in the terminal nodes display the incidence of the two species along with the lower and upper bounds of their 95% confidence intervals. Upper nodes show that the differences observed for the incidence values are significant for both species and sampling years at $P<0.05$.

Fig. 2 Comparison between the spore deposition rates of *Heterobasidion annosum* and *H. irregulare* in 2006 and 2016. For each sampling year the boxplot associated with the deposition rate (DR, spores·m$^{-2}$·h$^{-1}$) of both fungal species is reported. The boxes display the values ranging between the $25^{th}$ and $75^{th}$ percentiles, the horizontal thick line in between marks the median DR, the t-shaped whiskers outside the boxes identify the minimum and maximum values, while points over the whiskers’ bounds are outliers. Different letters indicate significant differences of the associated average values ($P<0.05$).

ranging between 70 and 89% (Table 2). Only 11% of the sampling points where the two fungal species occurred in coexistence in 2006 maintained such a state in 2016 (Table 2). Finally, the probability of a transition from the state Ha+Hi to the state of absence $\emptyset$ of the two species was 0% (Table 2).
The complete Markov matrix is reported in Table 2, along with the indication of the number of sampling points next to the canonical transition probabilities. The spatial distribution of the sampling points where 

\(H.\ annosum\) and \(H.\ irregulare\) were detected in 2006 and 2016 is shown with the corresponding transition state in Fig. 3. The row vector used for the Markov chain model was \(v = (0\%,\ 75\%,\ 6\%,\ 18\%)\), with the elements ordered as in the Markov matrix. The equilibrium was reached at \(k = 2\), corresponding to the year 2026. The following probabilities were associated with the different states at the equilibrium: 0% for Ha, 72% for Hi, 8% for Ha + Hi and 20% for Ø. The same probabilities were obtained up to \(k = 7\), confirming the stability of \(k\).

The common ratio \(c\) of the geometric progression modelling the past incidence of \(H.\ irregulare\) attained a value of 0.4. The intersection between the graph of the geometric progression and the detection threshold had its abscissa at year 1980, indicating the most likely estimate of the year when \(H.\ irregulare\) may have become established in the study site. The graphs of the lower and upper bounds of the CI95% crossed the detection threshold in points with abscissae at years 1958 and 1992, respectively. The overall incidence model of \(H.\ irregulare\), obtained by merging the results of the geometric progression, the spore

### Table 1

|          | 2006       | 2016       |
|----------|------------|------------|
|          | Ha         | Hi         | Ha + Hi    | Ø          |
|          | 4 (12.1%, 4.2–28.1% CI95%)a,A | 10 (30.3%, 15.7–48.4% CI95%)a,B | 9 (27.3%, 14.4–45.3% CI95%)a,A | 10 (30.3%, 15.7–48.4% CI95%)a,A |
|          | 0 (0%, 0–9.6% CI95%)b,A | 25 (75.7%, 57.8–88.3% CI95%)a,A | 2 (6.1%, 1.1–19.2% CI95%)b,B | 6 (18.2%, 8.2–34.5%)b,A |

Proportions are indicated along with their 95% confidence intervals. Lowercase letters refer to column-wise comparisons of sampling point frequencies among categories in the same sampling year, while uppercase letters are related to row-wise contrasts between sampling years within the same category. Different letters indicate significant differences of the associated values (\(P < 0.05\)). Ha: sampling points where only \(H.\ annosum\) was detected. Hi: sampling points where only \(H.\ irregulare\) was detected. Ha + Hi: sampling points where both \(H.\ annosum\) and \(H.\ irregulare\) were detected. Ø: sampling points where neither \(H.\ annosum\) nor \(H.\ irregulare\) were detected.

### Table 2

Markov matrix modelling the transition probabilities for the Markov chain model predicting the future trend in the incidence of \(Heterobasidion\ annosum\) and \(H.\ irregulare\)

|            | Ha2016 | Hi2016 | Ha2016 + Hi2016 | Ø2016 |
|------------|--------|--------|----------------|-------|
| Ha2006     | Pr(Ha2006→Ha2016) | Pr(Ha2006→Hi2016) | Pr(Ha2006→Ha2016 + Hi2016) | Pr(Ha2006→Ø2016) |
| 0%         | 4 (75%) | 10 (70%) | 9 (89%) | 10 (70%) |
| Hi2006     | Pr(Hi2006→Ha2016) | Pr(Hi2006→Hi2016) | Pr(Hi2006→Ha2016 + Hi2016) | Pr(Hi2006→Ø2016) |
| 0%         | 4 (75%) | 10 (70%) | 9 (89%) | 10 (70%) |
| Ha2006 + Hi2006 | Pr(Ha2006 + Hi2006→Ha2016) | Pr(Ha2006 + Hi2006→Hi2016) | Pr(Ha2006 + Hi2006→Ha2016 + Hi2016) | Pr(Ha2006 + Hi2006→Ø2016) |
| 0%         | 4 (75%) | 10 (70%) | 9 (89%) | 10 (70%) |
| Ø2006      | Pr(Ø2006→Ha2016) | Pr(Ø2006→Hi2016) | Pr(Ø2006→Ha2016 + Hi2016) | Pr(Ø2006→Ø2016) |
| 0%         | 4 (75%) | 10 (70%) | 9 (89%) | 10 (70%) |

Each cell shows the transition probability (\(Pr, \%\)) associated with any possible state transition (\(\rightarrow\)), as assessed from samplings conducted in 2006 and 2016. The corresponding number of sampling points is reported in brackets below the probability value. Ha: sampling points where only \(H.\ annosum\) was detected. Hi: sampling points where only \(H.\ irregulare\) was detected. Ha + Hi: sampling points where both \(H.\ annosum\) and \(H.\ irregulare\) were detected. Ø: sampling points where neither \(H.\ annosum\) nor \(H.\ irregulare\) were detected. \(Pr(S1 2006 \rightarrow S2 2016)\): transition probability from the state \(S1\) in 2006 (i.e. \(Ha_{2006}, Hi_{2006}, Ha_{2006} + Hi_{2006},\) or \(Ø_{2006}\)) to the state \(S2\) in 2016 (i.e. \(Ha_{2016}, Hi_{2016}, Ha_{2016} + Hi_{2016},\) or \(Ø_{2016}\))
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(A) Map of the study site (Sabaudia forest) showing the spatial distribution of the sampling points where *H. annosum* (panel a) and *H. irregulare* (panel b) were detected in 2006 and 2016. The transition map (panel c) shows the state transition of each sampling point between 2006 and 2016 as described by the Markov matrix. In the dot legend, the first state refers to 2006, the second to 2016. Acronyms of states included in the transition map are: Ha (sampling points where only *H. annosum* was detected), Hi (sampling points where only *H. irregulare* was detected), Ha+Hi (sampling points where both *H. annosum* and *H. irregulare* were detected), and Ø (sampling points where neither *H. annosum* nor *H. irregulare* were detected). Map coordinates reported along the x and y-axes are in WGS84/UTM projection system (m) (Gonthier et al. 2007), regional-scale migration from surrounding forests into the Sabaudia’s forest is likely to further contribute to an increase in *H. irregulare* population size.

It is noteworthy that while the distribution (i.e. number of sampling points with a positive *H. irregulare* detection), proportional representation (% of sampling points with a positive *H. irregulare* detection) and abundance (i.e. spore load) of *H. irregulare* increased when comparing 2006 to 2016 data, *H. annosum* displayed an inverse trend, with a significant decrease in its distribution, proportional representation and abundance. These results obtained using the entire Sabaudia’s forest dataset strongly suggest a competitive interaction is ongoing between the two species, with an outcome in favor of the alien pathogen. Similar results have been shown for other dual interactions among pathogens, such as, for instance, the interaction between *Ophiostoma novo-ulmi* and *O. ulmi* or between *Hymenoscyphus fraxineus* and *Hymenoscyphus albidus*, both ending in favor of the first species in each pair (Brasier and Bick 2001; McKinney et al. 2012).

Our analysis, like that of Kozanitas et al. (2017) who experimentally determined that coexistence of the two pathogens on the same wood substrate enhances the fruiting potential of the alien species without affecting that of the native one. We believe this to be one of the few examples in which spatially explicit resampling at several locations was employed to demonstrate species replacement of a native fungal species by an alien one (for other examples see McKinney et al. 2012; Kozanitas et al. 2017).

Although our experiment proves an increase in the spatial range of *H. irregulare*, an increase in its populations size, lack of a priori effects favoring *H. annosum* and its replacement by *H. irregulare*, the mechanisms leading to such expansion range, population increase and species replacement could be multiple. First, by producing a larger number of spores, *H. irregulare* is more likely to colonize newly available substrates such as snags and freshly cut stumps, thus reducing substrates available for its competitor. Second, by being a faster wood colonizer, *H. irregulare* may better utilize the available substrate than its competitor. Third, given that wood decay basidiomycetes are notoriously territorial through a process called somatic or vegetative incompatibility (Leslie 1993), the two traits above may lead to a direct antagonistic exclusion of *H. annosum* by *H. irregulare*. Although our data show that *H. annosum* is outcompeted by *H. irregulare*, the results of this study should not be used to infer an increased competitive advantage of *H. irregulare* over other wood-inhabiting fungi. In fact, two recent papers (Poloni et al. 2021; Pellicciaro et al. 2021) show that the North American *Heterobasidion* species can be controlled by stump applications of the saprobic competitor *Phlebiopsis gigantea*, as already reported for European *Heterobasidion* species (Garbelotto and Gonthier 2013).

The forward and backward modelling analyses, although performed using different methodologies and analytical approaches, are both robust and commonly used for demographic modelling (Verhulst 1977; Calter and Calter 2011; Dobrow 2016). Combined, the models predict an increase in the proportional representation of *H. irregulare* across the total number of points sampled in the Sabaudia forest. While Markov chain simulations estimate that the two interacting fungal species are likely to reach equilibrium in 2026, ten years from the second sampling, actual changes in proportional representation of the two species should be minimal between 2016 and 2026.
2026 and may be insignificant from a practical perspective. Forward simulations predict that by 2026 and then onward, 72% of the Sabaudia forest could be colonized by \( H. \text{irregulare} \), 8% might be colonized by both \( H. \text{irregulare} \) and \( H. \text{annosum} \), while 20% should be \textit{Heterobasidion} free. The persistence of \( H. \text{annosum} \), despite the asymmetrical fitness in favor of \( H. \text{irregulare} \), is to be expected and has been reported previously for other biological invasions involving one native and one alien organism characterized by asymmetrical fitness (Gurevitch and Padilla 2004; Downey and Richardson 2016).

The backward model indicated that the most likely arrival year of \textit{H. irregulare} at Sabaudia could have been 1980. That estimation of the arrival date may have a twofold application. First, it allows us to refine the regional scale prediction of spread rate in the highly fragmented and heterogeneous Roman countryside. Second, and more importantly, it allows to appraise the spatial spread in terms of number of hectares per year affected by pathogen in a single forest. Based on the data provided by this study and by the date and location of its original introduction, the regional scale spread rate of the pathogen in the Roman countryside should be 2 km/year, up from the previous estimate of 1.3 km/year. \textit{Heterobasidion irregulare} in a mixed oak-pine forest characterized by mild Mediterranean climate and good pine representation (Gonthier et al. 2012) is predicted to spread at an initial rate of 6–9 ha/year, reaching a maximum rate of 139 ha/year in the exponential phase of its spread. This is a significant spread rate, even at the beginning of the infestation, one that better exemplifies the actual threat that \textit{H. irregulare} poses to European forests. It is worth noting that forward and backward modelling analyses both hinge on field data collected at two time-points (i.e. 2006 and 2016), while both the past and the future scenarios span over several decades. Hence, our reconstructed and predicted scenarios necessarily include a level of uncertainty relative to the limited data available and to the assumptions underlying the models. Such assumptions include the stability of the probabilities embedded in the Markov matrix and the constancy of the common ratio of the geometric progression (Calter and Calter 2011; Dobrow 2016). Nonetheless, these and other assumptions are the backbone of modelling approaches across all scientific fields including biology, plant pathology, ecology, medicine, and geology (Dobrow 2016 and literature therein), in spite of the uncertainty inextricably associated with any modelling approach.

Finally, the issue of the \textit{H. irregulare}’s carrying capacity compared to that of \textit{H. annosum} remains
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Conclusions

By comparing the presence and abundance of each of two *Heterobasidion* species in the same sampling points ten years apart, this study provides the first direct evidence that the alien North American forest pathogen *H. irregulare* is invasive in Italy and is increasing both its geographic range and its population size, while locally replacing the native *H. annosum*. Although *H. annosum* is not predicted to completely disappear, its final frequency in Mediterranean mixed oak-pine forests invaded by *H. irregulare* will be low and its presence is predicted to always be in sympathy with the alien congeneric species. Replacement of *H. annosum* by *H. irregulare*, as documented by this study, suggests *H. irregulare* may become dominant even in pine stands, such as those of Central and Northern Europe, where incidence of *H. annosum* is already significant.

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Author contributions

MG and GL equally contributed to this work. MG: Conceptualization, Methodology, Investigation, Writing—original draft. GL: Conceptualization, Methodology, Data curation, Formal analysis, Software, Visualization, Writing—original draft. AVM: Investigation. PG: Conceptualization, Investigation, Methodology, Writing—review & editing, Supervision, Funding acquisition.

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Data availability

All data relevant to this work are provided as Online Resources.

Code availability

Can be provided upon request by the corresponding author.

Declarations

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funding sources of the research had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, and in the decision to submit the article for publication.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors have approved the contents of this paper and have agreed to the submission policies of Biological Invasions.

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