

Towards the cultivation of the bay boletus, *Imleria badia*: Primordia formation, mycorrhizal synthesis and geographic distribution

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Abstract

Aim of study: Here we take the first steps towards the cultivation of the gastronomically appreciated ectomycorrhizal fungus known as the bay boletus, *Imleria badia* (Fr.) Vizzini (2014). The cultivation of ectomycorrhizal species with their hosts presents a mycoforestry system in which food production and forestry can exist on the same parcel of land. We present results of attempts to synthesize *I. badia* mycorrhiza alongside an investigation into the distribution and climate tolerance of the species.

Area of study: The mycorrhizal-development component of the study takes place in laboratory and greenhouse facilities in Scotland, UK. The second component of biogeographic analysis covers the whole of Europe.

Material and methods: Strains of *I. badia* are sampled from forest sites in Scotland (UK), before being isolated in agar-based media within the laboratory. Mycorrhizal synthesis is attempted using the host tree species *Pinus sylvestris* L. in greenhouse conditions. Biogeographic analysis is then undertaken using global occurrence records from the Global Biodiversity Information Facility.

Main results: One isolated strain displayed the unusual characteristic of forming primordia in pure culture. Mycorrhizal synthesis occurred with the application of basidiospores, mycelia or a combination of both inoculation methods and identification was confirmed with PCR. Biogeographic analysis showed a broad distribution across Europe with some clear regional trends in seasonal fruiting patterns.

Research highlights: Successful mycorrhizal synthesis in greenhouse conditions of this gastronomically valued species presents the first step on the road to cultivation. A ready ability to produce primordia suggests high potential and biogeographic analysis allows informed selection of field-trial sites as the next step towards the goal of cultivation.

Keywords: mycelia; mycoforestry; mycorrhiza; PCR; primer; spore.

Hacia el cultivo del boleto bayo, *Imleria badia*: formación de primordios, síntesis micorrízica y distribución geográfica

Resumen

Objetivo del estudio: Este trabajo representa los primeros avances hacia el cultivo del hongo ectomicorrízico de interés gastronómico conocido como boleto bayo (*Imleria badia* (Fr.) Vizzini (2014)). El cultivo de especies ectomicorrízicas en asociación con sus árboles hospedadores constituye un sistema de micoforestación en el cual la producción alimentaria y la silvicultura pueden coexistir en una misma superficie. Se presentan resultados preliminares sobre la síntesis de micorrizas de *I. badia*, complementados con un análisis de la distribución y la tolerancia climática de la especie.

Área de estudio: El componente de desarrollo micorrícico del estudio se lleva a cabo en instalaciones de laboratorio e invernadero en Escocia, Reino Unido. El segundo componente, de análisis biogeográfico, abarca toda Europa.

Material y métodos: Se recolectaron fructificaciones de *I. badia* en bosques de Escocia (Reino Unido), que fueron aisladas en medios de cultivo a base de agar en el laboratorio. La síntesis micorrícica se realizó utilizando como especie hospedadora a *Pinus sylvestris* L. bajo condiciones de invernadero. Se realizó también un análisis biogeográfico empleando registros de distribución global procedentes del *Global Biodiversity Information Facility* (GBIF).

Resultados principales: Una de las cepas aisladas mostró la característica inusual de formar primordios en cultivo puro. La síntesis micorrícica se produjo con la aplicación de basidiosporas, micelio o una combinación de ambos métodos de inoculación, y la identificación fue confirmada mediante PCR. El análisis biogeográfico evidenció una amplia distribución de la especie en toda Europa, con patrones regionales definidos en la estacionalidad de la fructificación.

Aspectos destacados de la investigación: La síntesis micorrícica exitosa en condiciones de invernadero de esta especie de alto valor gastronómico constituye un avance significativo hacia su cultivo. La capacidad de generar primordios en cultivo puro refuerza su potencial de domesticación, mientras que el análisis biogeográfico proporciona información clave para la selección de sitios de ensayo en campo, como etapa subsiguiente hacia la meta del cultivo controlado.

Palabras clave: cebador; espora; micelio; micoforestación; micorriza; PCR.

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Introduction

The bay boletus is an edible, pored mushroom with a chestnut-coloured cap that is domed at first before flattening, with white flesh staining blue on bruising and eventually discolouring yellow after exposure to the air (Laessøe & Petersen, 2019). The common name refers to the colouration of the cap and this forest-dwelling species occurs with both conifer and broadleaved trees, is highly regarded by mushroom collectors and is often listed as a choice edible (Laessøe & Petersen, 2019; Muszyńska et al., 2020). As an ectomycorrhizal fungus (EMF), this species lacks photosynthetic capacity and is dependent on a host tree for carbohydrate supply, with which it forms specialised structures known as mycorrhizae to facilitate the mutualistic exchange of resources (Smith & Read, 2010). First described in 1818, the placement of the species was unsettled for a long time with disagreements between the *Boletus* and *Xerocomus* genera before multi-gene phylogenetic analysis suggested the placement as *Imleria badia* (Fr.) Vizzini (2014) (Vizzini (2014); Zhu et al., 2014). Widely distributed across Europe, this species is also recorded elsewhere in the world from America, Africa and Asia (Zhu et al., 2014; Muszyńska et al., 2020) although intraspecific divergence has been shown and specimens previously recorded in China as *Boletus badius* have been subsequently assigned as different *Imleria* species (Zhu et al., 2014).

With an aroma akin to the highly prized *Boletus edulis* Bull., *I. badia* is a widely appreciated and commercialised edible mushroom (Muszyńska et al., 2020). Often sold in a dried form, the species is also covered by European listings of commercialised fungi species (Peintner et al., 2013). As an easy to identify species with large and gastronomically appreciated fruiting bodies often occurring in high abundance, *I. badia* may be a strong candidate for cultivation. Mycoforestry describes the co-cultivation of such EMF species with trees, where the food-forestry land use conflict is mitigated and a food and forestry crop may

be achieved on the same parcel of land (Thomas & Vazquez, 2022; Thomas & Jump, 2023). Mycoforestry has great potential, but the first stage in cultivation is to create saplings dominated with mycorrhiza of the target species that can then be out planted for fruitbody production trials. This process can be challenging but the results can be impactful (Guerin-Laguette, 2021; Pérez-Moreno et al., 2021; Thomas & Vazquez, 2022; Yamada, 2022; Thomas & Jump, 2023). Mycorrhizal synthesis of *I. badia* has only been reported once before and under laboratory conditions, with the levels of successful mycorrhizal colonisation unreported (Duñabeitia et al., 1996). To develop cultivation, a methodology is first needed to create plants that are well colonised with the target species and under conditions that are scalable. Here we focus on this approach to attempt to synthesize mycorrhiza outside of the laboratory and within a protected growing environment. Production in a plant nursery-like environment is essential to facilitate scaling and out planting without transplant shock, and here we attempt this using basidiospores, mycelium or a combination of both as a source of inoculum. We assess the mycorrhization potential of these different approaches on the plant as a whole, but also at different root depths. The host tree we have chosen for this experiment is *Pinus sylvestris* L. Although *P. sylvestris* may acidify the soil, *I. badia* seems well adapted to such conditions with efficient micronutrient uptake in acid soils and perhaps retaining an advantage over other species in such conditions (Kottke et al., 1998). Additionally, although *I. badia* seems to associate with a broad range of tree species, there appears to be a particular affinity for pines (Laessøe & Petersen, 2019; Dyshko et al., 2024) and focussing our efforts on *P. sylvestris* allows application to commercial forestry systems.

Cultivation of EMF must also consider climate and soils as parameters that need to be understood to facilitate survival of the target species and eventual fruition. Within EMF cultivation this has been most extensively explored within the *Tuber* genus and has been shown to be pivotally important (Thomas & Büntgen, 2019; Barou et al., 2024; García-Montero et al., 2024). To inform the choice of sites for cultivation trials, such parameters also need to be explored for *I. badia*. As a first step towards this, we also present the results of an initial exploration of global occurrence records to help identify suited areas and climates for field trials that may follow successful mycorrhizal synthesis.

Material and methods

Culture conditions and mycorrhizal synthesis

Scots pine (*P. sylvestris*) seeds, obtained from Forestart (Forestart Ltd.), were surface sterilized by soaking in a 4% sodium hypochlorite solution for 5 minutes, followed by thorough rinsing with sterile distilled water. After sterilization, the seeds were air-dried under a laminar flow hood to prevent contamination. Seeds were sown on the 20th of April 2024 into a sterilised growth medium, which consisted of an autoclaved (128 °C, 1.5Mpa for 15 minutes) mixture of peat moss, perlite, and vermiculite (1:1:1, v/v/v) within a greenhouse. Prior to use, seedling germination trays were disinfected with 70% ethanol and 3% hydrogen peroxide to eliminate potential microbial contaminants.

Isolation of *I. badia* utilised tissue excised from the inner stipe of fresh fruiting bodies collected from woodland sites on the Isle of Bute, Scotland (UK). Fruiting bodies were surface sterilized using cotton moistened with 70% ethanol. Under a laminar flow hood, sections of approximately 0.5 cm³ from the inner stipe were carefully removed and placed onto Modified Melin-Norkrans (MMN, pH 5.5) media (Hung & Trappe, 1983) in petri dishes. Isolates were incubated at 23 °C and after two weeks, clean cultures were sub-cultured, with identity confirmed using EUR_x Direct Plant PCR kit with species-specific primers and as described below. Confirmed cultures were subsequently maintained at 23 °C with frequent rounds of sub culturing to produce enough material for inoculation experiments. Only one isolate was selected for inoculation trials, to keep treatments consistent between seedlings.

After three months of growth (30th of July 2024), seedlings were established enough to survive transplantation and the application of three inoculation treatments: inoculation with *I. badia* pure mycelium grown in MMN media, inoculation with *I. badia* basidiospores, and a combined treatment involving both mycelium and basidiospore suspension. For the mycelium treatment, at transplantation a 1 cm² agar plug containing actively growing fungal culture was gently massaged into the seedling root system before potting-on. In the basidiospore treatment, a vermiculite-basidiospore mix was massaged onto the root system during transplantation. For 20 plants, this mix consisted of 5 g of surface sterilized and dehydrated *I. badia* fruitbody caps – collected from the Isle of Bute (UK), dehydrated at approximately 35 °C and stored at 4 °C until use –

blended with 50 ml of distilled water and mixed into 5 g of autoclaved vermiculite. A subsample of this mix was used for each plant so that each seedling was exposed to approximately 0.25 g of dried fruitbody cap. Although spore loads were not calculated, this process ensured that treatments and basidiospore exposure remained relatively consistent between plants. In the combined treatment, both a 1 cm² mycelium plug and the basidiospore-vermiculite mix (as described above) were applied simultaneously. Each treatment was applied to 20 replicates of *P. sylvestris* seedlings. Following inoculation, all seedlings were re-potted into larger disinfected forestry plug trays (12 cm depth) filled with the same autoclaved plant-growth media to maintain semi-sterile growing conditions. The seedlings were watered regularly and exposed to natural diurnal fluctuations in irradiance and temperature within glasshouse conditions on the Isle of Bute (UK). Control plants underwent the same germination, husbandry and transplantation protocols but without the addition of spore or mycelial treatments.

Mycorrhizal analysis and primer design

Four months post-inoculation (November 2024), 10 seedlings from each treatment were randomly selected for analysis. The roots were carefully washed with tap water followed by sterile distilled water to remove any adhering debris. Root assessments were conducted at three distinct depths: 0–4 cm, 4–8 cm, and 8–12 cm. From each depth, three root segments were randomly collected and examined under a dissecting microscope to assess mycorrhizal colonization. The presence of *I. badia* and any other mycorrhizal contaminants was recorded to evaluate the extent and distribution of fungal colonization within the root system across different soil depths and logged as a percentile figure (as described by Thomas 2021). The identification of *I. badia* mycorrhiza was assessed following the methods described by Agerer (1991) and Agerer & Rambold (2004). Observed morphotypes were then sectioned for staining and further examination under a light microscope. This approach involved evaluating several characteristics, including mantle structure layers, cell shape, mycorrhiza length and structure, mantle sheath colour, overall shape, presence of rhizomorphs, hyphal organization, and Hartig net formation. Where these characteristics agreed with Agerer (1991), the mycorrhizae were assigned to *I. badia*. To assist in identification, some mycorrhizae were sectioned and stained with lactophenol cotton blue to enhance structural visualization. Statistical analysis of mycorrhiza counts was conducted to evaluate how treatment and soil depth influenced mycorrhizal colonization. Although a one-way ANOVA initially indicated a significant effect of treatment, checks using Levene's test ($F = 6.08$, $p = 0.0007$) and the Shapiro–Wilk test ($W = 0.731$, $p < 0.001$) revealed that the assumptions of normality and homoscedasticity were violated. Therefore, the ANOVA results were deemed invalid and to provide a robust analysis we exclusively used non-parametric tests (Kruskal–Wallis and Wilcoxon).

To further confirm identification, 9 mycorrhizae microscopically identified as *I. badia* were sampled from separate plants and stored at -20°C prior to molecular analysis. A pair of PCR primers specific for the ITS region of *I. badia* was designed using the Primer-Blast facility at NCBI (Ye et al., 2012). This uses Primer3 to design the primers and then BLAST and global alignment algorithms to screen primers against a user-selected database to avoid primer pairs that could produce non-specific amplifications. The ITS2 sequence ON962909.1 from *I. badia* isolate 5425_145 was used as the initial template and the “User Guided” Search Mode was selected to allow similar *I. badia* ITS2 sequences to be included as target templates. A primer pair producing a short amplicon (151bp) was selected from the options provided by the program. This meant that the primer pair was also suitable for real-time PCR. The primer pair sequences were: Forward primer TGTTGACATGGCTTGAGTTG. and Reverse primer AAGGCATCTGGGATTAGAAAGCA. For conventional PCR, the Extraction option of the EUR_x Direct Plant PCR kit was used according to the manufacturer's instructions but with some modification.

Prior to cycling, 9 individual mycorrhizae from separate plants and microscopically identified as *I. badia*, along with control non-mycorrhized root-tips were placed in separate 1.5 ml Eppendorf tubes. Direct DNA extraction was performed by adding 50 µl of EUR_x extraction buffer before incubation for 5 minutes at room temperature, then a further 50 µl of EUR_x dilution buffer was added before a further incubation of 5 minutes at room temperature. The samples were then centrifuged at 1500xg, and the supernatant containing the DNA was carefully transferred to a new tube for subsequent PCR analysis. The 20 µl PCR reaction was prepared by mixing 10 µl of x2 Master Mix (EUR_x/Direct plant PCR kit) with 1 µl of DNA extract, 0.5 µl of a 10 µM stock of each primer and 0.5 µl DNA polymerase with 7.5 µl of nuclease-free water. The reactions were denatured for 10 min at 98 °C, then amplified for 35 cycles of 98 °C, 10 sec; 65 °C, 20 sec; 72 °C, 20sec

before a final extension of 72°C for 60 sec. Samples were held at 15 °C at the end of the program. Following amplification, PCR products were visualized using 1% agarose electrophoresis gel to confirm the presence of *I. badia* DNA.

Mining and analysis of occurrence records

Occurrence data for *I. badia* (syn. *Xerocomus badius*, *Boletus badius*) was obtained from the Global Biodiversity Information Facility (GBIF). The dataset included geographic coordinates, scientific names, and event dates of recorded occurrences worldwide. The data was processed and analyzed using R (2024.12.0 Build 467) with the packages readr to read and clean the raw GBIF datasets. dplyr – for filtering, selecting relevant columns, and standardizing species names. Lubridate – to extract and classify occurrence dates into four quarters. Q1, Q2, Q3 and Q4. Sf (Pebesma, 2018), rnatualearth (South, 2017), and ggplot2 (Wickham, 2011) – to map the occurrence data over a high-resolution European basemap. Ggsplotial (Dunnington & Thorne, 2020) – to add a north arrow and scale bar for geographic reference. Occurrences were aggregated and visualized as color-coded points based on the assigned quarters, with point size reflecting the abundance of observations at each location. The filtering process included geospatial constraints, limiting the dataset to occurrences within European boundaries, and a temporal range from 1970 to 2025. Additionally, all basis of record categories were included to ensure comprehensive coverage of observation types. Further refinements were applied based on dataset source, publisher, IUCN Global Red List Category, issues, and flags to maintain data reliability.

Results

Mycorrhiza identification and primordia formation

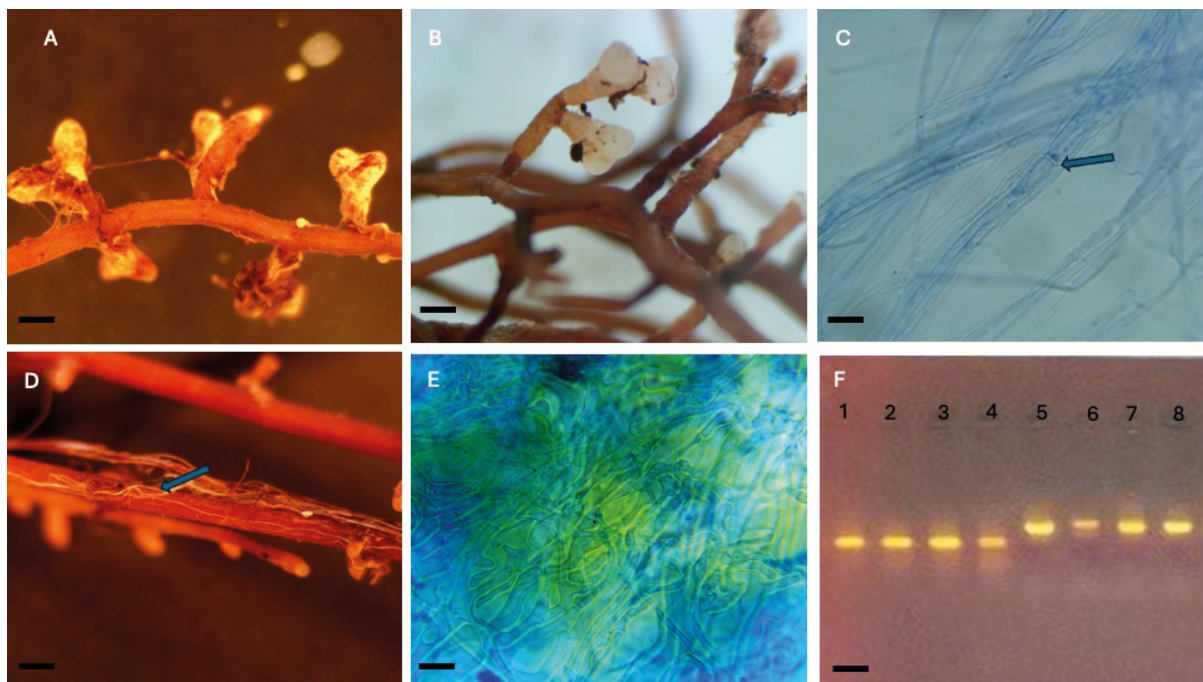


Figure 1. *Imleria badia* (Fr.) Vizzini (2014) mycorrhizae with *Pinus sylvestris* L., under dissection microscope (A,B), septate hyphae under a compound microscope showing dissolved septa (arrow) (C), rhizomorphs observed under dissection microscope (arrow) (D), mantle layers in plain view showing plectenchymatous organisation (E). Agarose gel electrophoresis (1% agarose) of PCR amplified products (F), lanes 1–4 are *I. badia* root tip isolates using species-specific primer sets, lanes 5–8 are the same samples using the ITS1/ITS2 generic primer set. Scale bars are 1 cm (A, B, D, F), 10 µm (C) and 20 µm (E).

Although at an early developmental stage, mycorrhizal structures were observed within the treatment plants and conformed to species descriptions provided by Agerer (1991) and Agerer & Rambold (2004). Mycorrhizae ranged from 2 mm to 10 mm in length (Figure 1) and either displayed a dichotomous ramification pattern or ramification was absent. The unramified ends varied in shape and colour, with younger sections appearing whitish, while older sections displayed ochre, yellowish-brown, or whitish hues. The mantle was either silvery or smooth, with infrequent emanating hyphae. Rhizomorphs were present and were whitish in colour (Figure 1). At the anatomical level, the rhizomorphs and emanating hyphae, lacked clamp connections (Figure 1) and the mantle displayed a plectenchymatous organisation. The Hartig net followed the Palmetti-type pattern. PCR with species specific primers confirmed the presence of *I. badia* within the observed mycorrhizae and by comparison, non-mycorrhized root-tips produced a negative PCR result.

Primordia formation was observed in two different media: MMN and previously on Potato Dextrose Agar (PDA). Cultures were maintained at 23 °C, and after subculturing, some cultures were left on the laboratory bench for several weeks where the temperature ranged from 14 °C to 18 °C. This shift to cooler conditions facilitated primordia development multiple times and, in both PDA, and MMN media (Figure 2), indicating that the decrease in temperature played a crucial role in triggering the differentiation process. These findings suggest that temperature modulation is a key factor influencing primordia formation in this system.



Figure 2. *Imleria badia* (Fr.) Vizzini (2014) fruiting body (A), primordia formation in PDA media (B), *I. badia* culture in MMN media (C), primordia formation in MMN media (D).

Mycorrhizal analysis

Within all treatment plants, all observed mycorrhizae were assigned to *I. badia*. The colonization percentage varied across different treatments and soil depths. Mycelial inoculation resulted in the highest colonization rates at 4 cm (11.7%), followed by a decrease at 8 cm (6.3%) and 12 cm (5.4%), with an overall average colonization of 7.8%. The basidiospore-only treatment showed colonization of 6.4% at 8 cm, while colonization at 4 cm and 12 cm returned lower figures (3.3% and 3.2%, respectively), with an overall average figure of 4.3%. The combination of basidiospores and mycelia showed the highest colonization at 8 cm (9.1%), followed by 4 cm (7.0%) and 12 cm (5.2%), leading to an average colonization of 7.16%. In contrast,

the control plants exhibited no *I. badia* mycorrhizae but some colonization with an unidentified species that was found across all depths (only in the control plants). Here the values are 1.11% at 4 cm, 1.33% at 8 cm, and 0.89% at 12 cm, resulting in an overall average of 1%.

An initial Kruskal–Wallis test ($\chi^2 = 26.77$, $df = 3$, $p = 6.57e-06$) with the different treatments and control group, confirmed that a significant difference was present. Pairwise Wilcoxon rank-sum tests (with Bonferroni correction) was then used to identify these differences and showed that mycelial ($p = 4e-06$), basidiospore + mycelial ($p = 0.00077$), and basidiospore-only ($p = 0.00031$) treatments significantly differed from the control. However, no significant differences were observed among the three inoculated treatments (all $p > 0.05$), suggesting that they have comparable mycorrhizal synthesis potential. To evaluate the role of soil depth, Wilcoxon comparisons across depths (4 cm, 8 cm, 12 cm) were conducted. No statistically significant differences were observed (4 cm vs. 12 cm, $p = 0.3497$; 8 cm vs. 12 cm, $p = 0.3668$; 8 cm vs. 4 cm, $p = 0.9994$), nor was there evidence of a depth $\times$ treatment interaction. This indicates that the effect of treatment on colonization remains consistent across depths.

By relying on non-parametric methods (Kruskal–Wallis and Wilcoxon), the analysis offers a statistically sound and assumption-free evaluation. These results confirm that all treatments are effective in establishing *I. badia* colonization, while soil depth plays no significant role (Figure 3).

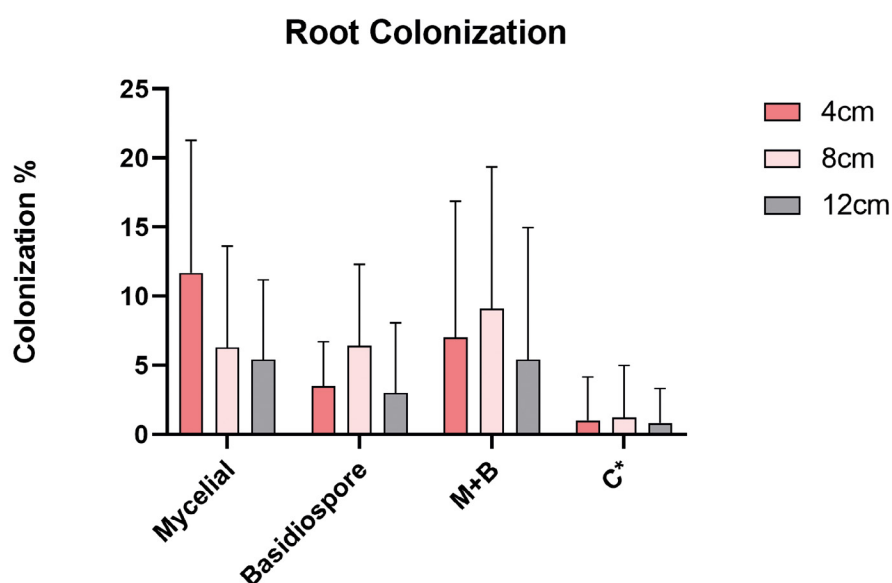


Figure 3. Root colonization percentage, according to the inoculation treatment as mycelial, basidiospore or combined mycelial and basidiospore. All mycorrhiza in treatment groups were assigned to *Imleria badia* (Fr.) Vizzini (2014) but a steric on the C (control) category denotes that mycorrhiza in this section were not *I. badia*, but were another unidentified species.

Occurrence records

Initially 69,737 global occurrences of *I. badia*, *X. badius*, and *B. badius* were retrieved from the Global Biodiversity Information Facility (GBIF) database (doi.org/10.15468/dl.tetd45). The dataset was then systematically filtered based on predefined criteria to refine the occurrence data for Europe (Figure 4). Following this rigorous filtering, a total of 34,151 observations remained and were subsequently used for the visualization and mapping of species occurrences across Europe. These refined data points provide insights into the spatial and temporal distribution of *I. badia* and its synonymous taxa across the continent.

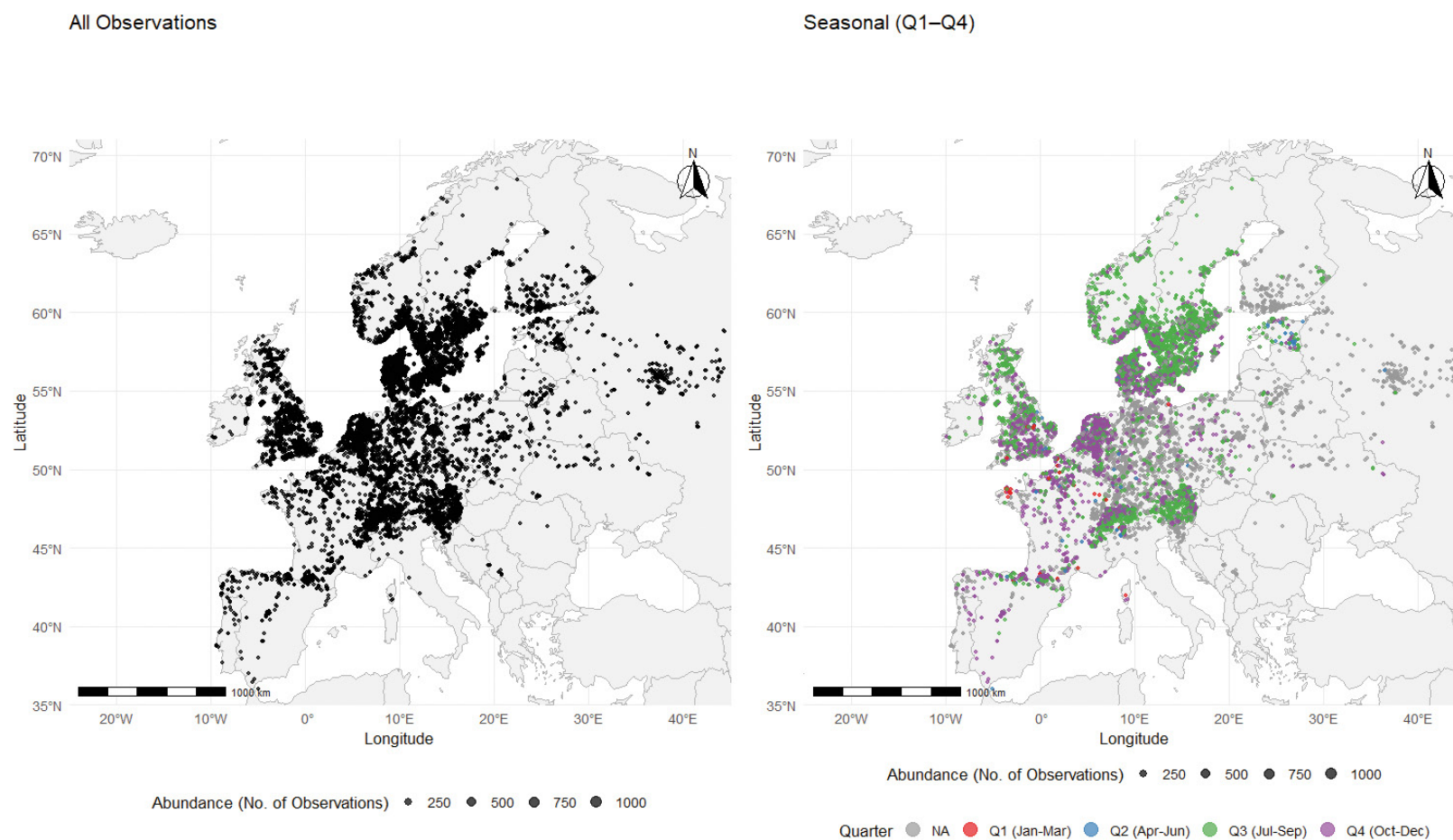


Figure 4. Maps displaying the general filtered European occurrence data (a) and the filtered European data according to the fruiting seasons across years 1970-2024 (b) for *Imleria badia* (Fr.) [Vizzini \(2014\)](#).

Discussion

Mycorrhizal development

To adapt any EMF species to mycoforestry, the first stage is to develop a methodology to produce mycorrhiza with a suited host plant (Guerin-Laguette, 2021; Pérez-Moreno et al., 2021; Thomas and Vazquez, 2022; Yamada, 2022; Thomas & Jump, 2023).

Mycorrhizal synthesis with *I. badia* has previously been reported to be successful with *Pinus radiata* D. Don., using both mycelial and spore inoculation (Duñabeitia et al., 1996), although the actual levels of mycorrhization were not reported. Other EMF show a mixed response to spore inoculation, with some species readily forming abundant mycorrhiza (De La Bastide et al., 1995; Nguyen et al., 2012; Lofgren et al., 2024) but others displaying low success rates and requiring mycelial inoculation for dependable mycorrhiza formation (Guerin-Laguette, 2021; Yamada, 2022). The results we present here agree with Duñabeitia et al. (1996), with successful mycorrhiza formation from both spore and mycelial inoculation. Going further, we quantified the mycorrhization level and found a range of between 2–28%, although the degree of mycorrhization did not differ across different root-zone depths. This agrees with data for another EMF species, *Tuber aestivum* Vittad. (1831), which also showed no variation by depth unless plants were exposed to the stress of simulated flooding (Thomas, 2021). Although the obtained mycorrhization rates are low, they show that mycorrhization synthesis is possible in glasshouse conditions. The inoculation protocol could be further improved to produce plants with higher colonisation rates that may be better suited to out-planting and in-field cultivation trials. Such processes of refinement have been achieved in other mycorrhizal plant production, such as for desert truffle cultivation (Morte et al., 2020). Further, it should be noted that inoculation occurred late in the growing season and the plants spent most time in cool weather prior to analysis. *I. badia* cultures grew well in the incubators at a temperature of 23 °C and so an extended development time, especially in warmer conditions will likely yield better mycorrhization rates. The addition of helper bacteria could also be beneficial as experiments with *B. edulis* have shown nearly a doubling of mycorrhization levels if the plants are also inoculated with *Pseudomonas fluorescens* Migula, 1895 (Mediavilla et al., 2016). Finally, an improved methodology should fully explore and develop plant husbandry alongside inoculation protocols, as these are both areas that are likely to yield immediate results.

Primordia formation

Primordia formation is the initial stage of the development of a fungal fruiting structure that can be visibly identified. Primordia formation of EMF in pure culture, independent of a host, is rare but has been reported in response to temperature cycling (McLaughlin, 1970), light conditions (McLaughlin, 1974) or exhausted nutrient sources within the growth media (Yamada et al., 2001). As the initial stage of fruitbody formation, primordia often terminate in pure culture but can develop further if the host plant is also present (Debaud & Gay, 1987) although they more likely form when mycorrhizae with young plants have been established and the plants are grown in substrate (Yamada et al., 2001). In a study of *Lyophyllum shimeji* (Kawam.) Hongo, isolates less than 10% produced primordia in agar, highlighting inter-strain variation (Ohta, 1994). Primordia in-cultures of *B. edulis* (Díaz et al., 2009) have previously been reported, but those of *I. badia* assigned as *X. badius*, have only been reported in pure culture just once before in a brief report—with no photography or details of the culture media (Pantidou, 1964). Our observations of primordia for *I. badia* occurred on two different media preparations and on plates that had not been fully colonised, so nutrient limitation was likely not a trigger but rather temperature cycling by cultures being moved for prolonged periods of time between incubators and the laboratory environment. Further, this occurred with just one strain which suggests that both temperature and strain selection are important for primordia formation. The development of primordia is of relevance as it suggests a ready ability of the strain to form fruiting bodies, which is a key requisite in mycoforestry systems.

Biogeography and suitability as a food source

To cultivate any species, an understanding of their biogeography is important. As a first step to help identify potential areas in which in-field trials can be attempted we mined 69,737 occurrence reports, before filtering to 34,151 usable geotagged European records of *I. badia*. The distribution shows a broad suitability across

Europe, from the mediterranean to the boreal biogeographic region of southern Finland. The geographic spread suggests a broad suitability across Europe, where the species occurs under a wide range of climate conditions. The distribution may also hint towards a unique resilience to climate change, as in a recent study of fungi collectors in Poland it was the only species that was found to be increasing in abundance, rather than declining, suggesting an increased ability to cope with drought conditions (Kotowski et al., 2021).

Going further, we were able to extract fruiting pattern data within the records and here we can see some key trends. In areas where summer rainfall is higher, such as Scotland, Norway and the mountain ranges of France and Spain – we see fruiting is most common in the summer quarter. Whereas areas with lower summer rainfall levels such as the Netherlands, England and the mediterranean – fruiting is more common in the autumn months. This data helps to plan in-field trials and to guide expectations of seasonal fruiting. Further, one anomalous cluster is found in northwestern France where there is a mass of fruiting records in winter. This latter cluster is undefined by temperature/precipitation and may be an indication of possible genetic variation within the population or even undescribed species such as those discovered previously within herbarium records in China (Zhu et al., 2014).

Finally, although *I. badia* is widely considered a choice edible with a long history of consumption (Laessøe & Petersen, 2019; Muszyńska et al., 2020), care must be taken in exploitation as a food resource as this species can bioaccumulate non-desirable compounds. For example, where soils are contaminated with lead and cadmium these compounds may reach levels within the fruiting body that are deemed unsafe for consumption (Malinowska et al., 2004). Collection, and therefore mycoforestry, should not be undertaken in areas where such pollutants exist. On the other hand, the value of this species as a food item is also emphasised by studies showing the high concentration of b-vitamins and antioxidants in the fruiting bodies themselves (Jaworska et al., 2015).

Conclusion

A gastronomically valued species that readily produces mycorrhiza from both spore and mycelial inoculation in a greenhouse environment, combined with an ability to form primordia in-vitro and a broad range of potential host plants present *I. badia* as a promising candidate for mycoforestry. Here we have presented methods to produce inoculated plants within a glasshouse environment, with suggestions for how to improve upon the processes tested. The next stage is to implement these improvements before moving to field trials. A broad occurrence distribution across Europe shows high suitability for the region with different fruiting patterns expected in different bioclimatic zones. The distribution data can be used to select sites suited to in-field trials although further exploration of suitable soil types is needed. Finally, this study lays solid foundations that can be built upon to further the goal of adapting *I. badia* to mycoforestry systems.

Data availability: Global occurrence data can be accessed from the Global Biodiversity Information Facility (GBIF) database (doi.org/10.15468/dl.tetd45).

Competing interests: The authors declare that: the data, arguments, and conclusions presented in the article are based on objective and independent scientific research, without any undue influence from Mycorrhizal Systems Ltd. or Professor Adrian Slater, Consultant.

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