Is the Perigord black truffle threatened by an invasive species? We dreaded it and it has happened!

Invasive alien species are species introduced deliberately or unintentionally to areas outside their natural habitats. They can cause a significant irreversible environmental and socio-economic impact at genetic, species and ecosystem levels, and as claimed by Moore (2000), ‘throughout the world, exotic aliens are wreaking havoc’. The control of these invasive alien species has been discussed at international conventions, such as the Bern convention in 1979. Research on biological introductions has primarily focused on plant and animal invaders. Until now only a few studies have dealt with the spread of fungi through different continents and countries (Desprez-Loustau et al., 2007), largely because of a lack of scientific knowledge of fungal biodiversity and ecology. Reports have mostly focused on the introduction of agronomically important plant pathogenic fungi (above all, rusts and Ascomycetes) and pseudofungi (Stramenopila and Peronosporomycetes), and their invasion routes (Desprez-Loustau et al., 2007). The most common way of introducing fungal pathogens is through the movement of infected planting stock or infested wood (Coetzee et al., 2001). The success of invasive fungal pathogens in these new environments might be explained by an increased aggressiveness towards new host species that have not had an opportunity to evolve resistance.

The ecological impacts of the intentional/unintentional introduction of fungal species, apart from plant pathogens, have been largely ignored (Schwartz et al., 2006). Regarding saprotrophic taxa, in-depth studies have only been carried out with a few species, for example Coprinopsis stangliana (Bougher, 2006), and some woodchip fungi (Shaw et al., 2004). As far as ectomycorrhizal fungi are concerned, the accidental introduction and spread of Amanita phalloides (Pringle & Vellinga, 2006), Boletus edulis s.l. (Hall et al., 1998) and Eucalyptus-associated species, such as Pisolithus spp. (Martin et al., 2002), have been carefully studied and monitored. These symbiotic fungi were accidentally introduced when allochthonous trees were planted for agriculture and reforestation (Hall et al., 1998; Martin et al., 2002). The deliberate movement of nonindigenous mycorrhizal fungal species and strains is a phenomenon occurring with increasing frequency as a consequence of the use of beneficial soil organisms to improve horticulture (Azcon-Aguilar & Barea, 1997), bioremediation (Leyval et al., 2002), reforestation (Duponnois et al., 2005) and edible fruit body production (Hall et al., 1998; Hall et al., 2003).

The impact of all these introductions on the overall native resident fungal communities is not well understood, and scant attention has been paid to the ecological impact of transporting nonpathogenic fungi across continents (with a few exceptions, e.g. Selosse et al., 1998). Monitoring efforts are therefore urgently needed to track the spreading pattern of introduced fungi.

Spreading the truffles: their distribution and genetic variability

Since the 1970s, humans have played an important role in the dissemination of some Tuber species, for example, by using inoculated seedlings to create artificial truffle grounds (Chevalier & Grente, 1979). One of the species most commonly inoculated is the famous Perigord black truffle (Tuber melanosporum Vittad.), which lives in symbiosis with several trees and shrubs. Besides its natural range (France, Italy and Spain), the Perigord black truffle is currently produced in Morocco, Australia and New Zealand, and it has recently been implanted in Canada, the USA, Argentina, Chile and Israel. However, in spite of this successful seedling production, the amount harvested annually has declined over the last century (Hall et al., 2003).

The name ‘truffle’ is a broad term that comprises fungal species of the symbiotic hypogeous genus Tuber, belonging to the Tuberaceae (Ascomycota, Pezizomycetes and Pezizales) family. Recently, Jeandroz and colleagues (2008) have highlighted the history and the biogeography of the Tuber genus, which seems to have originated in Laurasia during the Trias/Jurassic periods. Tuber currently displays a widespread geographic distribution over the northern hemisphere. It is present throughout all of Europe (Riousset et al., 2001), and five species have been found in North Africa (Khabzar et al., 2007). It is widespread in Asia (India, China and Mongolia) and is present in North America (Gilkey, 1954). The distribution of Tuber species depends on several factors: the distribution and migration of the host trees; dispersion by underground spores; dispersion via mammals; climatic conditions; and the existence of geographical barriers (Murat et al., 2004).

Bertault and colleagues (1998) found an extremely low level of polymorphism for T. melanosporum using random
amplification of polymorphic DNA (RAPD) and microsatellite analysis. Its low genetic diversity casts doubt on its survival when faced with climatic changes or invasive alien species. On the other hand, Bertault and colleagues (1998) claimed that there was no genetic structure for *T. melanoporum* because they did not find an increase in genetic differentiation with geographic distance using the Mantel test. A strong geographic pattern for *T. melanoporum* has instead been identified more recently (Murat et al., 2004) using moderate variations of the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS). None of the analyses, conducted using microsatellites and rDNA ITS sequencing, identified heterozygotes, suggesting that *T. melanoporum* has a very closed mating system, such as homothallism, pseudohomothallism or even exclusively selfing (Bertault et al., 1998; Murat et al., 2004). However, Paolocci and colleagues (2006) showed that recombination occurred in the white truffle *Tuber magnatum*; they explain the lack of heterozygosis by pointing out that the ‘DNAs extracted from the ascocarps are almost exclusively that of the maternal parent’. This hypothesis gives new ideas on the *T. magnatum* life cycle and opens the question of whether the same could be true for *T. melanoporum* (Rubini et al., 2007).

**Tuber melanoporum and Tuber indicum are close relatives**

The phylogenetic analysis of the genus *Tuber* showed that the Perigord black truffle is closely related to the Chinese truffle, *Tuber indicum* Cook & Massee (Wang et al., 2006). Moreover, both species are morphologically similar and confusion between them is possible (Paolocci et al., 2000; Riouset et al., 2001). Unlike *T. melanoporum*, *T. indicum* has a high level of genetic diversity, and a strong phyleogeographic structure has recently been identified in China (Wang et al., 2006). The natural production of this species is high (at least 300 tons in 2006, according to Knapp, 2006). Even though the total production is increasing each year, the local production is declining, mostly as a result of destructive harvesting methods. For example, before 1993 more than 20 tons were harvested in Huidong county (Sichuan, China) whereas only 4–5 tons were recorded as being harvested in 2003 (Wang et al., 2007).

Importation of the Chinese truffle, instead of the Perigord truffle, for sale in Europe has been known about since the middle of the 1990s and, as claimed by Hannah Beech (TIME Magazine, April 17, 2005): ‘Chinese fungi are flooding gourmet-markets, and Europeans are not amused’. However, *T. indicum* does not have the same organoleptic qualities as its European cousin, which can cost 1000 Euro kg$^{-1}$. Because of the absence of specific regulations in France and Spain, *T. indicum* can be sold in these countries, unlike in Italy where the sale of *T. indicum* is not permitted (law n°752, December 16, 1985, modified May 17, 1991). *In vitro* experiments have displayed that *T. indicum* is dominant, competitive and more aggressive than *T. melanoporum* (Gérard Chevalier and Alessandra Zambonelli, pers. comm.). It is therefore of concern that *T. indicum* could be used (mixed with *T. melanoporum*) to inoculate seedlings, which could thereafter be disseminated in truffle ground and then threaten the indigenous *T. melanoporum* populations (Paolocci et al., 1999; Mabru et al., 2001). To investigate the accidental introduction of *T. indicum* in Europe, molecular tools for discriminating both are already available (Paolocci et al., 1999; Mabru et al., 2001).

**From China to Piedmont**

The potential introduction of *T. indicum* received its first experimental confirmation during a survey in a truffle plantation in Piedmont (Italy), where by chance we identified the Chinese black truffle, *T. indicum*, in addition to the expected Perigord black truffle. In this plantation the owner introduced hazel and hornbeam seedlings in 1997 that were expected to be inoculated with *T. melanoporum*, but he never harvested any truffle fruiting bodies. To establish the presence of *T. indicum*, we randomly sampled root tips and soil samples from six trees (five hornbeams and one hazel) in the plantation (for methodology details see the Fig. 1 legend). The molecular analysis, based on the rDNA ITS sequencing, enabled us to identify two *Tuber* species (*Tuber borchii* and *T. indicum*) within genotyped ectomycorrhizal tips (data not shown). In a second step, fungal DNA was extracted directly from the soil samples and specific primers were used to genotype *T. melanoporum* and *T. indicum*. The Perigord black truffle was identified in two soil samples, and *T. indicum* was identified in three soil samples (data not shown). The four *T. indicum* sequences obtained from the Piedmont mycorrhizae and soil samples were identical and clustered in the *T. indicum* clade II (Fig. 1). This clade comprises all the samples from Gong-shan (Yunnan), Miyi and Panzhihua (Sichuan) (Wang et al., 2006). As a result of the high phyleogeographic structure of *T. indicum*, we suggest that the fruit bodies used to inoculate the seedlings were probably imported from these regions of China. The present report is therefore the first evidence that *T. indicum* has been used to inoculate seedlings implanted in Europe and that this species can spread in European ecosystems. *Tuber indicum* is a novel invasive alien species in Italy. More than a gourmet-market problem, it constitutes an ecological threat for the Perigord truffle, in particular because *T. indicum* can be found in China in ecosystems that are similar to those in central Italy (Wang et al., 2007).

**Truffles: indigenous vs alien species**

The replacement of *T. melanoporum* in productive plantations with autochthonous species, such as *Tuber brumale* Vittad. and *Hebeloma* sp., has been well documented (Chevalier et al., 1982; Granetti & Angelini, 1992). One of the main reasons
for these substitutions is the low competitiveness of *T. melanosporum* regarding these species. This trait is probably one of the reasons for its progressive decline. However, the ecological consequences of the introduction of *T. indicum* into a *T. melanosporum*-producing area in Europe are still unknown and their close phylogenetic relationship opens new questions of scientific relevance.

- Are both species able to inbreed?
- Is *T. indicum* able to spread over long distances?
- Is *T. indicum* really capable of replacing *T. melanosporum* in truffle grounds?

Our findings point out the need for local agencies to control not only the importation of *T. indicum* fruiting bodies but also the production of mycorrhizal sceddings. Moreover, it is of vital importance to draw attention to *T. indicum* as an invasive alien species in Europe and to prevent its dissemination in *T. melanosporum* production areas. It is recommended that *T. melanosporum* plantations in France, Italy and Spain are investigated for the presence of *T. indicum*. How can we control or eliminate *T. indicum* in Europe? In France, Gérard Chevalier advised pulling out trees and disinfecting soils. However, this is a difficult option, particularly because owners have invested money in their plantations, and this option may need to be a political decision, perhaps with incentives for the owners, such as financial support.

In conclusion, we would like to point out the importance of conserving truffle biodiversity, above all for species such as *T. melanosporum* that has a low genetic diversity and, like other fungi (Gange et al., 2007), might be influenced by climatic changes (Bertault et al., 1998).

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