Edible ectomycorrhizal mushrooms: challenges and achievements[1]

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Abstract: There are about 2500 recorded species of edible mushrooms. The most expensive and sought after of these mushrooms belong to the mycorrhizal group and include *Tuber melanosporum* Vitt. (Périgord black truffle), *Tuber magnatum* Pico & Vitt. (Italian white truffle), *Tricholoma matsutake* (Ito & Imai) Sing. (matsutake), *Boletus edulis* Bull: Fr. sensu lato (porcini) *Cantharellus cibarius* Fr.: Fr. (chanterelle), and *Amanita caesarea* (Scop.: Fr.) Pers: Schw. (Caesar's mushroom). The total market for these is measured in billions of US dollars. Over the past 100 years, harvests of many mycorrhizal mushrooms have declined dramatically, which has prompted interest in the development of methods for their cultivation. So far only a few species of truffles have been produced in commercial quantities, although methods have been developed that may see the cultivation of species such as *Cantharellus cibarius*, *Lyophyllum shimeji* (Kawam.) Hongo, and *Lactarius deliciosus* (L.: Fr.) Gray. Despite this, many of the most expensive mycorrhizal mushrooms, including *Tuber magnatum* Pico & Vitt. and *Tricholoma matsutake*, have defied cultivation. Our paper will attempt to highlight possible reasons why mycorrhizal mushrooms have proven to be so difficult to grow and how we might better manage mycorrhizal mushroom forests to sustain natural production.

Key words: edible, ectomycorrhiza, mushroom, truffle.

Résumé : Il y a environ 2500 espèces de champignons reconnus comme comestibles. Les plus recherchés et les plus chers appartiennent au groupe des mycorhiziens, et incluent les *Tuber melanosporum* Vitt. (truffe noire du Périgord), *Tuber magnatum* Pico & Vitt. (truffe blanche d'Italie), *Tricholoma matsutake* (Ito & Imai) Sing. (matsutaké), *Boletus edulis* Bull : Fr. sensu lato (cèpes) *Cantharellus cibarius* Fr. : Fr. (chanterelle) et *Amanita caesarea* (Scop. : Fr.) Pers : Schw. (amanite des César). Le marché mondial pour ces champignons se mesure en milliard de dollars US. Au cours des cent dernières années, la récolte de plusieurs espèces de champignons comestibles a chuter très fortement, ce qui a encouragé la recherche de méthodes permettant leur culture. Jusqu'ici, seulement quelques espèces de truffes ont été produites en quantités commerciales, bien que des méthodes aient été développées qui pourraient conduire à la culture d'espèces telles que les *Cantharellus cibarius*, *Lyophyllum shimeji* (Kawam.) Hongo et *Lactarius deliciosus* (L. : Fr.) Gray. Malgré tout, plusieurs des champignons mycorhiziens dispendieux, tels que les *Tuber magnatum* Pico & Vitt et *Tricholoma matsutake*, demeurent toujours un défi à la mise en culture. Les auteurs tentent de mettre en lumière les raisons qui pourraient expliquer pourquoi les champignons mycorhiziens sont tellement difficiles à cultiver, et comment on pourrait mieux développer l'aménagement des champignons mycorhiziens comestibles venant en forêt, de manière à assurer leur durabilité.

Mots clés : comestible, ectomycorhize, champignon, truffe.

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Introduction

More than 2500 fungi are known to produce edible mushrooms (Chandra 1989). Edible ectomycorrhizal mushrooms (EEM) comprise a specific group of edible fungal species that form symbiotic associations with their host plants (Smith and Read 1997). This includes about 200 common edible ectomycorrhizal mushroom species that are widely eaten in the Northern Hemisphere, although many more, particularly in Africa and South America, have yet to be recorded (e.g., Boa 2002; Wang et al. 2002). A few species have well-established worldwide markets in excess of US$2 billion, while many others are locally important (Olivier 2000; Hall et al. 2003*a*). EEMs are not only gourmet foods, they are also a livelihood for many people who collect the mushrooms from their natural habitats or cultivate them (Molina 1998; Boa 2002). *Tricholoma matsutake* (Ito & Imai) Sing. (matsutake) in Japan, and truffles such as *Tuber melanosporum* Vitt. (Périgord black truffle) and *Tuber magnatum* Pico & Vitt. (Italian white truffle) in France and Italy, are also an important part of the culture (Ogawa 1978; Trappe 1990; Hall et al. 1994), while others have medicinal properties (Ying et al. 1987; Wang 1995; Xu 1997).

Production decline

Unlike saprobic edible mushrooms, with few exceptions, the market of EEMs is supplied from what can be harvested

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from natural forests. Unfortunately, harvests of many edible mushrooms have declined over the past century, because of changes in their natural environment caused by various natural and social factors (Arnold 1991; Chevalier 1998; Reyna et al. 2002; Hall and Wang 2002). For example, *Tuber melanosporum* harvests have fallen from around 2000 t in the 1900s to sometimes less than 100 t annually (Olivier 2000; Hall and Wang 2002). Similarly, current matsutake production in Japan is just 5% of 1940s harvests (Ogawa 1978; Wang et al. 1997).

The decline in the availability of EEMs and increased demand have encouraged research into developing technologies for the cultivation of EEMs as well as methods for the sustainable productivity in natural forests. Despite numerous scientific publications and the establishment of thousands of hectares of plantations, the downward trend in EEM production continues (Ogawa 1978; Hosford et al. 1997; Wang et al. 1997; Chevalier 1998; Hall and Wang 2002; Reyna et al. 2002; Hall et al. 2003*b*; Pilz et al. 2003).

Cultivation

Currently only *Tuber melanosporum* and *Tuber uncinatum* have been cultivated commercially (Chevalier 1998, 2002; Olivier 2000; Hall and Wang 2002), although some success has been achieved with *Lactarius deliciosus* (L.: Fr.) Gray, *Lyophyllum shimeji* (Kawam.) Hongo, *Tuber borchii*, *Tuber formosanum*, *Rhizopogon rubescens*, and *Terfezia laveryi* (Iwase 1997; Honrubia et al. 2002; Wang and Hall 2002; Zambonelli et al. 2002; H.-T. Hu, National Taiwan University, Taiwan, personal communication). Host plants have also been infected under sterile conditions in the laboratory or greenhouse with *Boletus edulis* Bull: Fr. sensu lato (porcini), *Cantharellus cibarius* Fr.: Fr. (chanterelle), *Lactarius hatsutake*, and *Lactarius akahatsu*, although fruiting bodies have not yet been produced in the field. (Wang et al. 1998*b*; Danell 2002; Yamada et al. 2002). However, despite many years of study, attempts to cultivate species such as *Tricholoma matsutake* and *Tuber magnatum* have met with failure (Bencivenga 1998; Wang and Hall 2004).

The management of plantations for better EEM production is an important issue, and over the years a large body of knowledge has accumulated. However, we still do not know why some truffières consistently produce large quantities of truffles, while others produce nothing (Chevalier 1998; Gregori 2002; Hall and Wang 2002; Reyna et al. 2002). Obviously a greater understanding of this and other species of EEMs is needed.

Hypogeous edible mushrooms

Périgord black truffle and other Tuber

Attempts to grow truffles began in the 18th century when Joseph Talon in France and Francesco Francolini in Italy began the cultivation of truffles. They grew seedlings around black truffle (*Tuber melanosporum*) trees and transplanted them to new areas, where eventually these seedlings also produced truffles (Chevalier 1998; Hall and Wang 2002; A. Zambonelli, University of Bologna, Bologna, Italy, personal communication). However, it was not until the early 1970s that methods were devised in Europe for infecting suitable host plants with black truffle spores under controlled conditions and routinely producing truffles in artificial truffières (Chevalier 1998; Olivier 2000). Since then the technique has been extensively used in European countries, with the largest Périgord black truffle truffière, some 500 ha, established in Spain. On average, 2–50 kg/ha are produced without irrigation and up to 150 kg/ha with irrigation (Chevalier 1998; Olivier 2000; Hall and Wang 2002; Reyna et al. 2002; A. Zambonelli, University of Bologna, Bologna, Italy, personal communication), although in France, a truffière is considered successful if 10 years after planting 50% of the trees produce truffles and yields reach 15–20 kg/ha (Chevalier 1998). Similar technology has also been used to successfully grow the Burgundy truffle (*Tuber uncinatum*; Chevalier and Frochot 1990, 1997; Chevalier 2002) in France, *Tuber borchii* (Zambonelli et al. 2002) in Italy, and desert truffles, such as *Terfezia claveryi*, in Spain (M. Honrubia, University of Murcia, Murcia, Spain, personal communication).

In the 1980s, many Périgord black truffle truffières were established in the United States. In 1988 and 1993, two truffières, one in northern California and one in North Carolina, began producing truffles (Garland 1996; Hall and Wang 2002).

In the early 1980s, research into the cultivation of the Périgord black truffle and other EEMs began in New Zealand, with the first seedlings produced in 1986 and the first truffières established in 1987. Since then, more than 100 truffières have been established in both the North and South Islands. In 1993, the first Périgord black truffles in the Southern Hemisphere were harvested from a truffière on the east coast of the North Island. Between 1997 and 2002, this 0.5-ha truffière produced between 9 and 65 kg truffles per year. So far, 7 of the 11 truffières established prior to 1990 have produced truffles, all of which are in the warmer parts of the country (Hall and Wang 2002). In 1993 the first black truffle truffières were established in Tasmania, Australia, and the first truffles harvested in 1999. The island now has more than 50 truffières. We understand that 6 of them have produced small quantities of truffles. Truffières have also been established in New South Wales, Victoria, and Western Australia, and plantations now amount to a total of 90 ha (Cooper 2001). A truffière in Western Australia began production in 2003.

Another example of the introduction of *Tuber melanosporum* to a previously truffle-free region is in Israel, where a small quantity of truffles was harvested from a 7-year-old truffière in 2000 (Pinkas et al. 2000; Kagan-Zur et al. 2002).

In Asian countries, *Tuber indicum* and closely related species have been collected and traded for centuries (Trappe 1990; Wang and Hall 2001), but it was not until the 1990s that research on the cultivation of truffles in Asia began. The first successful cultivation of a *Tuber* species in Asia was *Tuber formosanum* in a truffière established in 1989 that produced truffles in 1996 (H.-T. Hu, National Taiwan University, Taiwan, personal communication). Recently, research has also begun on the cultivation of *Tuber melanosporum* and Chinese truffles in China (Chen 2002; Gong et al. 2003; T. Zhuning, Institute of Forest Ecology, Hunan Forestry Academy, Changsha, China, personal communication).

Experimental plots of *Tuber uncinatum* have been established in Sweden and in New Zealand (Wedén and Danell 2002). Several small truffières of *Tuber borchii* have also been established in New Zealand. To date, infections have spread on new roots, although fruiting bodies have yet to be harvested (Hall and Wang 2002).

Desert truffles

Desert truffles, species of *Terfezia* and *Tirmania*, are used as foods in Africa and the Middle East (Trappe 1990). They form different kinds of mycorrhizas, such as endomycorrhiza, ectomycorrhiza, or ectendomycorrhiza, with *Helianthemum* and other members of Cistaceae (Awameh 1981; Fortas and Chevalier 1992; Kagan-Zur 1998; Honrubia et al. 2002; Gutierrez et al. 2003). Mycorrhizas of desert truffles have been produced in semiaxenic culture and in vitro (Awameh 1981; Fortas and Chevalier 1992; Morte and Honrubia 1995; Kagan-Zur 1998; Honrubia et al. 2002). Plantations were established in Spain in 2000, and truffles were harvested just 2 years later. Yields of desert truffles from natural bushes typically range from 50 to 170 $kg \cdot ha^{-1} \cdot year^{-1}$ in Spain. However, production from irrigated truffières (90 $L \cdot m^{-2} \cdot year^{-1}$) has been as high as 300 kg/ha, suggesting a potential for a very profitable EEM industry in semidesert areas of warm countries (M. Honrubia, University of Murcia, Murcia, Spain, personal communication).

Shoro

Shoro (*Rhizopogon rubescens*) is a delicacy in Japan, and it has been successfully cultivated in Japan since the late 1980s (Iwase 1997; K. Iwase, Biological Environment Institute, KEEC Center Co., Ltd., Tokyo, Japan, personal communication). Three of four shoro (*Rhizopogon rubescens*) plantations established in New Zealand in 1999 have all produced fruiting bodies (Wang and Hall 2002).

Epigeous edible ectomycorrhizal mushrooms

Compared with truffles, cultivation of epigeous EEMs has been less successful than for the hypogeous species, partly because of the relatively small resources applied. So far, only saffron milk cap (*Lactarius deliciosus*) in New Zealand (Wang and Hall 2002) and shimeji (*Lyophyllum shimeji*) in Japan have been successful. The first saffron milk cap fruiting body was produced in 2002 in a North Otago *Pinus radiata* plantation just 18 months after planting. A year later, the plantation produced the equivalent of 50 kg of fruiting bodies per hectare (Wang et al. 2003). Poitou et al. (1984) reported that fruiting bodies of *Lactarius deliciosus* were produced on inoculated *Pinus pinaster* seedlings in a nursery, while seedlings of *Pinus massoniana* infected with *Lactarius hatsutake* in a nursery in Hunan, China, produced fruiting bodies in 2001, 3 or 4 years after inoculation (T. Zhuning, Institue of Forest Ecology, Hunan Forestry Academy, Changsha, China, personal communication).

In Nara and Kyoto Prefectures, Japan, fruiting bodies of shimeji were produced from artificially infected seedlings in 1998 and 1996 (K. Iwase, Biological Environment Institute, KEEC Center Co., Ltd., Tokyo, Japan, personal communication).

Fruiting bodies of *Cantharellus cibarius*, *Lactarius deliciosus*, *Lactarius akahatsu*, and *Lyophyllum shimeji* have been produced on inoculated seedlings growing in open pots in a greenhouse (Guerin-Laguette et al. 2000*a*; Yamada et al. 2002; Danell 2002; Ohta 1994), and Ohta (1994, 1998) was able to produce fruiting bodies of *Lyophyllum shimeji* from pure cultures (Yoshida and Fujimoto 1994; Kawai 1997). However, experimental plots of *Cantherellus cibarius* in Sweden so far have failed to produce fruiting bodies, even though the infections have spread onto new roots (Danell 2002). Similarly, *Boletus edulis* and *Amanita caesarea* (Scop.: Fr.) Pers: Schw. (Caesar's mushroom) infections on *Castanea sativa* seedlings persisted on 90% of infected plants (Meotto et al. 1999). Less success has been achieved with plants infected by *Lactarius sanguifluus* and *Tricholoma matsutake*, because infections failed after outplanting (Eto 1990; Hu 1994; Wang 1995; Wang et al. 1997; Guerin-Laguette et al. 2000*b*; Yamada et al. 2002; Gonzalez-Ochoa et al. 2003).

Production of infected seedlings

The major challenge facing the scientist trying to cultivate EEMs is the method for producing stable infections on suitable seedlings. The "dirty technology" used by Joseph Talon and Francesco Francolini in the early 1800s is the oldest of the techniques (Hall et al. 2001; Hall and Wang 2002). Although it has been largely superseded by more modern methods, it is still being used in Spain (S. Reyna, Polytechnic University of Valencia, Spain, personal communication) and has been used to produce matsutake-infected pine trees in Japan (Iwase 1997), Korea (Lee 1988), and China (Wang 1995). However, spore inoculation remains the most popular method and has been used successfully to infect trees with many common hypogeous edible mycorrhizal fungi, with the notable exception of *Tuber magnatum* (Bencivenga 1998). While this method has also been used to produce trees infected with some epigeous edible mycorrhizal fungi (Tan Zhuming, Institute of Forest Ecology, Hunan Forestry Academy, Changsha, China, personal communication), generally the starting point is the preparation of mycelial cultures. Regrettably, success with such techniques has been limited despite its large-scale application in forestry (Grove and Malajczuk 1994).

Appropriate selection of suitable host-plant species is essential for successful infection (Olivier 2000). For example, Chevalier (1998) found that for the cultivation of Périgord black truffles, *Quercus pubescens* and *Quercus ilex* were better than hazels (*Corylus* spp.), and *Corylus colurna* was better than *Corylus avellana*, and plants raised from cuttings and tissue cultures produced truffles only 5 years after planting. More research on the relative advantages of different host species in different ecological areas is certainly needed (Olivier 2000), as is the possibility of superior strains of the fungi.

The quality of infected seedlings is also a vital component in the cultivation of EMM. In France, a strict procedure for controlling the quality of infected seedlings has been adopted by a number of nurseries (Olivier 2000), although it is not enshrined in potent legislation. Reyna et al. (2002) suggested that 250–500 infected root tips, equivalent to 10%–25% of the root, was an acceptable level of infection, while Italian mycologists believed that about 33% was acceptable (Alessandra Zambonelli, University of Bologna,

Bologna, Italy, personal communication). The general opinion is that contamination should never be higher than 25%. However, sampling roots from Melfert bags, a commonly used container, is difficult, because many mycorrhizas break off, making estimates of percent infection difficult. Furthermore, morphological identification of infections of different *Tuber* species is sometimes difficult or impossible, and molecular methods are expensive and, therefore, currently impossible to apply on a large scale (Mabru et al. 2001). Consequently, the accurate identification of inoculum before inoculation and limiting contamination during incubation of the inoculated plants are essential parts of the production procedure. Regrettably, such care is not always taken, and poorly infected plants are widespread.

At the Hiroshima Prefecture Forest Experimental farm, Gumu, *Pinus densiflora* seedlings were successfully infected using the so-called secondary infection method (Ogawa and Ito 1989). This involved planting 3- or 4-year-old pine seedlings close to a matsutake shiro, and then, once infected, transplanting them into nonproducing areas. Despite considerable research, only one fruiting body was ever produced in this way in the second year after the shiro formed. These methods have also been adopted in Korea and China but so far without success (Lee 1988; Park et al. 1995; Wang 1995; Koo and Miliek 1998; Gong et al. 1999).

Inoculating existing mature trees with black truffle

Reyna et al. (2002) believe that commercial harvesting of EEMs has removed the opportunity for new trees or roots to become infected by spores and that unless remedial work is undertaken the existing natural truffle-producing forests in Spain will lose most of their productive capability in the next 20–30 years. To correct this, they devised a method for inoculating existing mature trees by producing root traps in the drip zone of trees and then inoculating the soil with spores. The results showed that black truffle infections successfully established on the new roots. Spores to inoculate existing trees have also been used in attempts to replace *Tuber brumale* with *Tuber uncinatum* in France (Frochot et al. 2001) and to inoculate pine trees with saffron milk cap in Spain (M. Morcillo, Micologia, Forestal & Aplicata, Barcelona, Spain, personal communication). Similar attempts to use this method in Japan to inoculate existing pine trees with matsutake spores have not been successful (Ogawa 1978; Ogawa and Ito 1989).

Establishing and managing plantations

Over the past 200 years, considerable knowledge and experience have been accumulated on establishing and managing Périgord black truffle truffières (Chevalier 1998; Olivier 2000; Hall et al. 2001), with climate probably the single most important factor. For example, in New Zealand only truffières north of Christchurch have produced truffles (Hall et al. 2001). Soil conditions are also important and more amenable to modification than climate. For example, adding lime to acidic soils to raise the pH can make them suitable for the cultivation of the Périgord black truffle (Chevalier 1998; Olivier 2000; Garland 2001; Hall et al. 2001). The physical soil characteristics are perhaps even more important, because the Périgord black truffle requires good drainage (Chevalier 1998; Olivier 2000; Hall et al. 2001).

Management methods of truffières vary from intensive procedures (the Pallier method) to minimal management (the Tanguy method) (Chevalier 1998). The Pallier method, adapted from orchard management, includes soil tillage, irrigation, weed control, and tree pruning. It is expensive, but yields are generally good, whereas the Tanguy method tends to leave things to chance. Irrigation is important for the Périgord black truffle (Hall et al. 2001), the desert truffle (Honrubia et al. 2002), and the saffron milk cap (Wang et al. 2003). Soil physiology and biology (microflora, microfauna, and mesofauna) might also be important, but our knowledge is very limited (Chevalier 1998; Olivier 2000; Hall et al. 2003*b*).

Sustainability of edible ectomycorrhizal mushrooms

Recreational collecting

Collection of ectomycorrhizal and other edible mushrooms in native forests for food and recreation is a popular activity in many cultures and countries (Hall et al. 2003*a*). When the harvest of edible mushrooms is recreational, rather than a source of income, generally there is little impact on the environment (Arnold 1991; Egli and Ayer 1998; Pilz et al. 2003). However, large-scale commercial harvesting, which generates significant income, can cause serious environmental problems in forests, especially when some commercial harvesters disrespect both the mushrooms and their forest environment (Pilz et al. 2003). As a consequence, the management of commercially harvested areas has become a major concern.

Commercial harvesting

Harvesting, processing, and trading EEMs from native forests, such as truffles in Europe (Olivier 2000) and matsutake in Asia (Wang et al. 1997) and North America (Hosford et al. 1997), is a multimillion dollar industry probably generating over US$2 billion/year on worldwide markets (Hall and Wang 2002). In many areas this makes significant contributions to regional economies and to the income of harvesters. For example, harvesting, processing, and exporting matsutake in South Korea and China generates US$20–80 million/year to rural populations. China alone has exported more than 500–600 t of matsutake per year to Japan since the 1990s, with Yunnan exporting 350 t in 1997 worth US$40 million (= RMB$3200 million). Because the Chinese matsutake-producing areas are all located in poor, remote, mountainous regions, revenue generated by harvesting matsutake is a particularly important source of income, even though the retail price is well in excess of the relatively low export price. In Zhong-dian County, Yunnan, 80% of farmers are involved with commercial harvesting, processing, and matsutake trading, an industry that can generate up to RMB$50,000 – 60,000 annually per family, which represents up to 20 times a normal farming family's annual income (Gong et al. 1999). Canada, the USA, and Mexico also export 500–700 t of white matsutake (*Tuber magnivelare*) to Japan each year (Hosford et al. 1997; Redhead 1997). While the income from this is relatively small, it is a locally important source of income.

Management and conservation of forests producing edible ectomycorrhizal mushrooms

Conservation

Most EEMs are found growing in remote mountainous regions where logging and harvesting EEMs are important sources of food and revenue to local people. Under the pressure of hunger and cold, deforestation and overharvesting are common. Environmental deterioration has been the result and the reason for EEM species becoming endangered or disappearing from some areas. Therefore, conservation of existing EEM environments is an urgent matter, particularly in developing countries. In China, commercial logging in the southwest region has been banned since the late 1990s, and a small number of matsutake natural reserves have been established for study and conservation (Winkler 2002; Y. Wang, data not shown). However, problems will not subside until governments, local authorities, and scientists work together to put in place sustainable management practices for environmental conservation. To help with this, international assistance and cooperation are urgently required (Boa 2002).

Asian matsutake forests

Following Japan's newfound prosperity in the 1950s, many *Pinus densiflora* forests were abandoned, and as a consequence the litter layer built up and the forests became denser and unsuited to matsutake. Insect pests also became established, which wiped out much of the *Pinus densiflora*, so that now production of *Tricholoma matsutake* has declined dramatically and disappeared from some regions (Ogawa 1978; Iwase 1997). In contrast, in South Korea matsutake forests have been well protected and harvesting and trading of the mushroom well managed (Wang 1995). Even so, in South Korea, too, the production of matsutake has declined by 7% since the 1980s (Koo at al. 2001), and the host forests have passed through the "golden age" of matsutake production, as matsutake host plants are slowly replaced by trees in the next stage in the succession. Regrettably, in many parts of China, similar problems exist and might only be solved when substantial collective efforts are made by government, law makers, harvesters, and scientists to address the required management practices (Gong et al. 1999; Wang and Hall 2004).

Following the disappointing research into the cultivation of *Tricholoma matsutake* in the 1970s and early 1980s, Ogawa and Ito (1989) and Tominaga and Komeyama (1987) turned their attention to developing management techniques for maximizing *Tricholoma matsutake* production in existing forests. This was achieved by reducing the thickness of the litter layer, thinning, removing competing ectomycorrhizal fungal fruiting bodies, protecting *Tricholoma matsutake* forests from diseases, insects, birds, and other animals; and inoculating the soil by spraying on *Tricholoma matsutake* spores or retaining some of the mature fruiting bodies on the forest floor (Ogawa and Ito 1989; Wang et al. 1997; Gong et al. 1999; Wang and Hall 2004).

North American matsutake and other edible ectomycorrhizal mushrooms

In the 1980s, commercial harvests of mycorrhizal and other edible mushrooms from native forests created many social and environmental problems and generated considerable public concern (de Genus 1995; Hosford et al. 1997; Redhead 1997; Pilz and Molina 2002). In the Pacific Northwest region of North America these problems were abated after refinements were made to harvest regulations and harvesters were educated in the environmental impacts of overharvesting (de Genus 1995; de Genus and Berch 1997; Pilz et al. 2003). Current scientific evidence suggests that harvesting is unlikely to have short-term detrimental effects on the resources of the Pacific Northwest, although research on the long-term cumulative impacts of commercial harvesting is still needed (Pilz and Molina 2002).

New sources and conservation

New sources of edible ectomycorrhizal mushrooms from developing countries

Compared with developed countries, the range of species present and their market value in developing countries are poorly understood and only rarely appear on Western markets (Boa 2002; Ducousso et al. 2002; Flores et al. 2002; Liu 1998). For example, it was not until the 1990s that poorly understood truffles from east Asia began to appear on international markets, even though they had probably been gathered and sold in China for centuries (Tao and Liu 1989; Trappe 1990; Wang and Hall 2001). E. Horak estimated that there are at least 1500 mushroom species in Yunnan province, many of which are edible but have yet to be studied (P.G. Liu, Kunming Institute of Botany, Kunming, China, personal communication). Similarly, Petersen and Zang (1986) found three new species of *Ramaria* in a wild mushroom market in Yunnan, China, and matsutake has only recently been found in Mexico, Nepal, and Bhutan (Wang and Hall 2004). The situation in Africa is no better, being termed a mycologists' desert (Boa 2002), although research programmes are being established (Boa 2002; Flores et al. 2002).

Basic research

Taxonomy of edible ectomycorrhizal mushrooms

Taxonomic and nomenclatural problems in *Tuber* were recently reviewed (Trappe 2001). Molecular tools have helped solve some of the taxonomic problems and our understanding of the species concept in *Tuber* (Amicucci et al. 1998, 2002; Mabru et al. 2001) and other genera (Bergius and Danell 2000; Moor et al. 2002), but many problems still remain. For example, Asiatic species of black truffles on European markets (Oliver 2000; Mabru et al. 2001) are all referred to as *Tuber indicum*, although *Tuber sinense*, *Tuber himalayense*, *Tuber pseudohimalayense*, and *Tuber formosanum* are also found in southwest China, Taiwan, and Japan and probably appear in European markets (Hu 1992; Wang et al. 1998*a*; Wang and Hall 2001; Yamanaka at al. 2001). The species from southwest China, which can easily be mistaken for *Tuber melanosporum*, have great intraspecific heterogeneity, even between ascocarps collected in the same field (Wang and Hall 2001). Consequently, it is likely that several undescribed species are present in these areas. Similar problems also exist in the taxonomy of *Boletus edulis* and other species (Wang et al. 1995; Moor et al. 2002), and large numbers of "new" species that are consumed in developing countries — species for which we don't even have scientific names!

Trophic states of "ectomycorrhizal" edible fungi

Traditionally fungi have been assigned to three categories: parasites, saprobes, or symbionts. However, there is a continuous gradation of behaviour rather than sharp boundary lines between these fungal groups, with environment affecting the nature of an association (Lewis 1973; Garrett 1960; Hall et al. 2003*b*; Smith and Read 1997). Mycorrhizas are often considered to be examples of mutualism, but there appears to be a continuum of plant responses to mycorrhizal colonization ranging from positive to neutral to negative. Bidartondo et al.'s work (2002) also suggests that mutualisms are not stable end points in evolution but are inherently unstable and can be disrupted by "conflicts of interest" between the partners, with breakdowns leading to parasitism or even the complete dissolution of the symbiosis (Hibbett 2002; Hibbett et al. 2000). Pellmyr et al. (1996) suggested that this host or partner shift is a mechanism that can provide a route of evolutionary escape among obligate symbionts. Leake et al. (2003) reviewed the interactions between mycorrhizal and saprobic mushrooms and noted that although they share and compete for carbon and other nutrients during nutrient cycling within the ecosystem, the interactions between mycorrhizal mushrooms in natural ecosystems remain obscure.

Mycorrhizal fungi might be considered parasitic on plants when the net cost of the symbiosis exceeds net benefits, that is, when the growth increment brought about by the increased uptake of nutrients is exceeded by the drain on the plant's carbohydrate imported by the fungus. Parasitism can be developmentally induced, environmentally induced, or genotypically induced (Johnson et al. 1997).

Tricholoma matsutake is a good example of the "breakdown of mutualisms". Even though it has traditionally been considered a typical ectomycorrhizal fungus (Ogawa 1978; Yamada et al. 2002), evidence suggests that it can be parasitic or saprobic at least for part of its life cycle or under some environmental conditions where the host plant is stressed (Masui 1927; Hiromoto 1963*a*, 1963*b*; Ogawa 1978; Wang 1995; Wang et al. 1997; Vaario et al. 2002). For example, matsutake hyphae can invade cortical cells and sometimes completely destroy them, particularly in the laboratory, and chlamydospores can be produced inside cortical cells, suggesting pathogenicity (Figs. 1, 2), whereas its colonization of substrates in the shoro is more typical of saprobic mushrooms such as shiitake (*Lentinus edodes*) (Wang 1995). It is, therefore, hard to accept that matsutake is a typical or normal ectomycorrhizal fungus; indeed this may well be the reason why all efforts to maintain infections on seedlings have failed (Ogawa 1978; Wang 1995; Iwase 1997; Yamada et al. 2002). Even typical ectomycorrhizal fungi, such as *Tuber melanosporum*, will kill all vegetation surrounding the host tree producing the brûlé (Hall et al. 2001), while *Lactarius deliciosus* and *Thelephora terrestris* can invade host or grass root cortical cells (Figs. 3, 4). In contrast, Ohta (1994) has found that mature fruiting bodies of the ectomycorrhizal fungus *Lyophyllum shimeji* can be produced in pure culture, either with or without the presence of host plants.

Tripartite relationships

Mycorrhizal associations do not exist alone in the soil, as there are often other organisms associated with them (Hall et al. 2003*b*). Bacteria, particularly *Pseudomonas*, are often found associated with mycorrhizas (Garbaye 1994; Kropp and Mueller 2000; Poole et al. 2001) and fruiting bodies of *Cantharellus cibarius* (Danell et al.1993). A range of culturable bacteria and other microorganisms were also found associated with *Tuber borchii* mycorrhizas (Sbraba et al. 2002), while *Mortierella* sp. is often found within the fruiting bodies and shiro of *Tricholoma matsutake* (Ogawa 1978; Wang 1995).

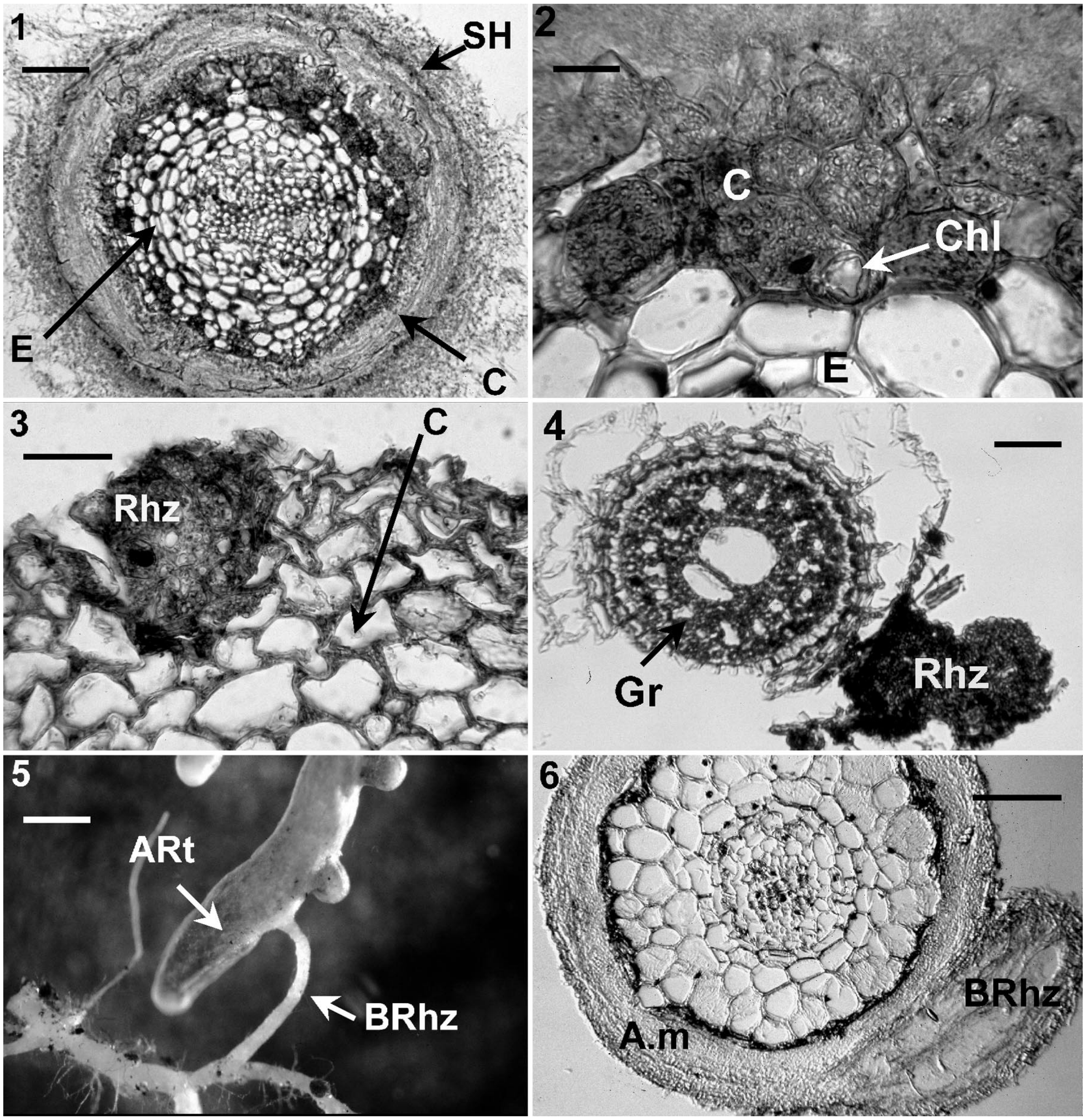
In natural forests, *Tricholoma matsutake* infections are often found combined with other mycorrhizal infections on the same root or replaced by another infection. Wang found that matsutake-infected roots can be covered by hyperparasitic, dark-brown hyphae (Wang 1995), and Terashima (1993) found several mycorrhizal fungi coexist with multiple infections on roots in *Tricholoma bakamatsutake* shiros. Similarly, Agerer (1991) and other mycologists found mixed mantles of two mixed mycorrhizal species. These multiple infections may create more potential for connection between trees, soil water, and nutrients, which may have biological and ecological importance, in particular, to the association of matsutake with its host plants. Because matsutake infections are especially aggressive, the simultaneous presence of extensive beneficial mycorrhizal fungi on a root system might perhaps limit any detrimental effects caused by matsutake.

Boletus edulis, *Amanita muscaria*, and *Amanita excelsa* grow together in the same locations in New Zealand and other countries (Hall et al. 2003*b*). On closer investigation, it was found that rhizomorphs of *Boletus edulis* were often closely wound around *Amanita* root tips, forming composite mycorrhizas (Figs. 5, 6). Nutrients may flow between the two species, which might be necessary for fructification of *Boletus edulis*, but further investigation using molecular immunological tools is needed.

Molecular technology

In recent years, molecular techniques have been used to accurately identify EEMs and their isolates, detect infections, and monitor the development of infections in soils. For example, Bergius and Danell (2000) recognized that European matsutake, *Tricholoma nauseosus*, is conspecific with Asian *Tricholoma matsutake*, based on DNA sequencing of the rDNA ITS region, an issue that has long confounded morphological taxonomy of this fungal group. A rapid molecular typing method for reliable detection of Asian black truffle species has also been developed to identify fruiting bodies and mycorrhizal infections (Mabru et al. 2001). PCR (polymerase chain reaction) methods were devised for the detection of *Boletus edulis* and other species of *Boletus* and *Suillus* in foods (Moor et al. 2002). Molecular technology also provides a new approach for understanding mycorrhizal associations that will improve our understanding of methods that need to be employed for their cultivation and the management of plantations (Franken and Requena 2001; Martin 2001; Nehls et al. 2001; Hall et al. 2003*b*).

Figs. 1–6. Fig. 1. Cross section of pine root infected with *Tricholoma matsutake* showing hyphae invading and destroying cortical cells. C, cortex; E, endodermis; SH, sheath hyphae. Scale bar = 0.2 mm. Fig. 2. Cross section of pine root infected with *Tricholoma matsutake* showing chlamydospores produced in cortical cells, suggesting pathogenicity. C, cortex; E, endodermis; Chl, chlamydospore. Scale bar = 50 μm. Fig. 3. Cross section of *Pinus radiata* root showing *Lactarius deliciosus* rhizomorph invading the root cortical cells. C, cortex; Rhz, rhizomorph. Scale bar = 40 μm. Fig. 4. Cross section of grass root showing *Lactarius deliciosus* rhizomorph invading root cortical cells. Gr, grass root; Rhz, rhizomorph. Scale bar = 50 μm. Fig. 5. *Boletus edulis* rhizomorph found in close association with *Amanita muscaria* mycorrhizal root tip, forming a composite mycorrhiza on *Pinus radiata*. ARt, *Amanita muscaria* mycorrhizal root tip; BRhz, *Boletus edulis* rhizomorph. Scale bar = 1 mm. Fig. 6. Cross section of the *Pinus radiata* composite mycorrhiza showing the *Amanita muscaria* mantle and *Boletus edulis* rhizomorph. A.m, *Amanita muscaria* mantle; BRhz, *Boletus edulis* rhizomorph. Scale bar = 0.2 mm.



Conclusion

Edible mycorrhizal mushrooms are not only a gourmet food but also a source of income for those who cultivate or collect them from the wild. The most expensive and sought after edible mushrooms belong to this group, for example, *Tuber melanosporum*, *Tuber magnatum*, and *Tricholoma matsutake.* Over the past 100 years, harvests of many mycorrhizal mushrooms have declined dramatically, which has prompted interest in the development of methods for their cultivation. So far only a few species of truffles have been produced in commercial quantities, although methods have been developed that may see the large-scale cultivation of other species, such as *Cantharellus cibarius*, *Lyophyllum shimeji*, and *Lactarius deliciosus*. Most significantly, many of the most expensive mycorrhizal mushrooms, including *Tuber magnatum* and *Tricholoma matsutake*, have defied cultivation.

Some technologies have been developed for the cultivation of a few EEM and management of existing EEM-producing forests to maximize their production. Unfortunately, these efforts do not seem to have had an effect on diminishing production. The exploitation of new sources of EEMs, especially in developing countries, will provide income for these countries and would find ready markets in developed countries. However, overpicking has to be avoided, and conservation and management are needed if EEM are not to be damaged. It is expected that mycologists facing these issues will experience considerable challenges in years to come.

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