# Chapter 13 Truffle Cultivation in China

**Xianghua Wang** 

# 13.1 Current Status

Compared with Europe and North America, China has a much shorter history of scientific research on truffles. Truffles have been harvested by local people in China for many years, but the first scientific report of the genus *Tuber* in the country occurred late in 1980s. Mycologist B. Liu from Department of Biology, Shanxi University, gave the first record of truffles in China, *Tuber taiyuanense* B. Liu from Xishan, Taiyuan (the capital of Shanxi Province, after which the truffle was named) (Liu 1985). This small spiny-spored truffle, which has been placed in the Rufum clade (Wang et al. 2007), did not address commercial interests. In 1989 and 1992, respectively, *Tuber sinense* K. Tao & B. Liu was described as a new species and *Tuber indicum* Cooke & Massee was recorded from China for the first time (Tao et al. 1989; Zang et al. 1992). These two black truffles, which are highly similar to *Tuber melanosporum* Vittad. in appearance, motivated subsequent research in China on truffle ecology, taxonomy, phylogeny, and mycorrhizal relationships. Research on the cultivation of Chinese truffles followed.

García-Montero et al. (2010) gave a detailed review of research on truffles in China, which also mentioned progress on cultivation on truffles. Here we will not repeat the general content in the review, but will focus on more detailed introduction and discussion on the issues related with cultivation of truffles in China. Three parts will be included in this chapter: current status, review on relevant technology and knowledge, and advantages, challenges, and future research directions.

Truffle (*Tuber*) cultivation is a new topic in China. The earliest attempt to cultivate truffles in China started in 1987 in Taiwan (Hu et al. 2005). Six-monthold seedlings of *Cyclobalanopsis glauca* (Thunb.) Oerst. were inoculated with a

X. Wang (🖂)

Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R. China e-mail: xhwang@mail.kib.ac.cn

A. Zambonelli and G.M. Bonito (eds.), *Edible Ectomycorrhizal Mushrooms*, Soil Biology 34, DOI 10.1007/978-3-642-33823-6\_13, © Springer-Verlag Berlin Heidelberg 2012

spore suspension of *T. formosanum* nom. inval. (a small phylogenetic clade in *T. indicum* s.l.) collected in central Taiwan in 1987. One year later, the seedlings were transplanted into bigger containers and kept for another year in greenhouse before transplanting to the field. Two years before the transplantation, a site, later was used as truffle land, was limed with mixture of  $CaCO_3:MgCO_3$  (1:1, mol/mol). In 1997, 8 years after the transplantation, the first fruiting occurred in the truffière, and the next year, ca 10-kg truffles were harvested from the 0.6 ha of truffière. This was the first successful cultivation of truffles in China.

Using similar methods and under the direction of H. T. Hu, researchers from Guizhou Academy of Forestry, Guiyang, in cooperation with a company, started cultivating *T. indicum* s.l. in 2002 in central Guizhou, a place with subtropical climate and moderately high elevation 1,000–1,500 m (Hu et al. 2010). Seedlings of two indigenous tree species (i.e., *Quercus aliena* Bl. and *C. glauca*) were inoculated by a spore suspension of *T. indicum*  $(1.6 \times 10^5/\text{ml})$ . *Tuber* mycorrhizae formed 120 days after inoculation. One and a half years after the inoculation, the seedlings were transplanted to the field. Soil at this site is classified as yellow soil with a pH between 5.0 and 5.5. Before transplantation, the soil was limed with quicklime. In winter of 2008, five years after the transplantation, more than 1.5-kg truffles were harvested from some of seedlings of *Q. aliena* and one seedling of *C. glauca*. This is the first report of successful cultivation of truffles in mainland China.

The first report of successful cultivation of exotic truffle species in China was from Guizhou in 2008. Also cooperating with H. T. Hu from Taiwan, a company established a plantation of *T. melanosporum* near Guiyang (Longli County). At the end of 2008, the first yield, 1.0-kg ascomata of *T. melanosporum* were harvested from the truffière (Gong 2009). Although detailed information on the establishment process is lacking, it is likely similar to that of *T. indicum* and *T. formosanum*, since the truffière is at the same site as *T. indicum* s.l. mentioned above and is operated by the same research group and company.

There are several other truffle plantations established by different institutions in China, though they have not fruited yet. All of these plantations are situated in subtropical China. They include a 2-ha plantation in Cili County, northwestern Hunan, established by Hunan Academy of Forestry in 2001. This is a plantation of *T. melanosporum* established using inoculum introduced from France (Anonymous reporter 2009; Tan and Fu 2003); more than 130 ha plantations in Panzhihua, Sichuan Province, established by Panzhihua Academy of Agriculture and Forestry under the direction of researchers from IPLA (Istituto per le Piante da Legno e l'Ambiente, Italy) during 2006–2008 (Lin et al. 2008); two plantations near Chuxiong, Yunnan, established by Chuxiong Institute of Forestry and Yunnan Academy of Agriculture; five plantations near Kunming, Yunnan, established by Kunming Institute of Botany, Chinese Academy of Sciences, and Yunnan Academy of Agriculture, respectively, during 2008–2011; 2–3 plantations near Bijie, Guizhou, established by Kunming Institute of Botany, Chinese Plantations near 3–6 years old.

To our knowledge, all of the seedlings used for these plantations were produced in China. Every institution which established the plantations above has their own truffle nursery. The biggest nursery is owned by Panzhihua Academy of Agriculture and Forestry, Sichuan. In cooperation with IPLA, the academy produced almost 100,000 truffle seedlings in 2007, using the tree hosts *Castanea mollissima* Bl., *Pinus armandii* Franch., and *Pinus yunnanensis* Franch. (Lin et al. 2008). Hunan Academy of Agriculture owns a nursery that is able to produce 10,000 seedlings of *T. melanosporum* annually. This might be the biggest *T. melanosporum* nursery in China. Among the institutions producing truffle seedlings, two of them have registered and published the technology in State Intellectual Property Office of P.R. China. Although one of them has received an authorized patent (ZL200810058447.9), up to now there is no supply of commercial seedlings that are qualified by commercial standards in China. With the amount of truffle seedlings increasing year by year, strict quality assurance and control are becoming urgent in China.

## **13.2** Review of Relevant Technology and Knowledge

## 13.2.1 Taxonomy

Only black truffles have been cultivated in China. There are three groups of indigenous black truffles in the country: T. indicum group, Tuber pseudohimalayense G. Moreno et al. group, and Tuber aestivum Vittad. group, most of which are found from southwestern mountains, with elevation 1,500-2,500 m. Seven names have been used for the species of the three groups: T. aestivum, T. formosanum, Tuber himalayense B. C. Zhang & Minter, T. indicum, T. pseudohimalayense, Tuber pseudoexcavatum Y. Wang et al., and T. sinense. Among them, T. aestivum, a famous culinary truffle in Europe, was reported from the country very recently (Chen et al. 2005; Song et al. 2005). This black truffle is commercialized in southwestern China but with much less yield than T. indicum s.l. The Chinese material named as "T. aestivum" shows minor morphological differences from European material (Chen et al. 2005; Song et al. 2005; Zambonelli et al. 2012), but the close relationship between them is obvious. There is no report on the cultivation of Chinese "Burgundy truffle." Considering the high culinary value of European T. aestivum/uncinatum and close relationship between Chinese and European material, this Chinese counterpart is a logical candidate for cultivation.

*Tuber pseudoexcavatum*, a black truffle with excavated ascomata and spinoreticulate spores, is another truffle popularly commercialized and exported to Europe. It has similar commercial value as *T. indicum* group but has yet been cultivated. Mycorrhizal synthesis with this species on the European oak species *Q. ilex* L. has been reported by García-Montero et al. (2008). *Tuber pseudoexcavatum* was recently found to be conspecific with *T. pseudohimalayense* and should be treated as a synonym of the latter (Chen and Liu 2011; Manjón et al. 2009). In China, the truffle is found under *P. yunnanensis*, which suggests a wide distribution in the Yunnan-Guizhou Plateau. There is interest in cultivating this species in China.

The truffles popularly cultivated in China belong to the *T. indicum* complex. It is this group that has stirred most debate and discussion both in China and Europe during the past two decades. Although many names have been applied to species in this complex (e.g., *T. formosanum*, *T. himalayense*, *T. indicum*, and *T. sinense*), researchers have reached a consensus that there are two separate phylogenetic species (clades) in the complex (Chen et al. 2011; Paolocci et al. 1997; Roux et al. 1999; Wang et al. 2006; Zhang et al. 2005). Since it is hard to find reliable morphological characters to define the two clades, the specific name that should be applied to each clade is still pending. The biggest barriers are the fact that morphological variations within one clade overlap those within the other clade, and up until now, sequences from type specimen are lacking (Chen et al. 2011).

Hu et al. (2005) used T. formosanum as the target species for cultivation. This is a black truffle which is included within the *T. indicum* B clade (Chen et al. 2011; Huang et al. 2009) and may represent a small geographical variety of the species in Taiwan. This truffle associates with C. glauca. It is the first truffle that was successfully cultivated in China. Chen (2003) also used ascomata of T. formosanum for mycorrhizal synthesis, but there is no subsequent report whether fruiting ever occurred. The Chinese truffles used by other researchers for cultivation or mycorrhizal synthesis are merely labeled as T. indicum, without further classification (Chen 2003; Geng et al. 2009; Hu et al. 2004, 2010; Lin et al. 2008). Since there was no detailed analysis on the identity of truffles used as inoculum, it is not clear which phylogenetic species (clade A or B) was used. According to our own experience, ascomata used for inoculations often come from markets and could have diverse origins; consequently, a mixture of the two species is most likely used for inoculations. Taking cost of identification into account, it is reasonable to believe that cultivation of T. indicum in China includes both species without critical sorting and may even include the unintentional inclusion of T. pseudohimalayense/ pseudoexcavatum.

Besides black truffles, species of Magnatum clade, Gibbosum clade, and Puberulum clade also have high culinary value (Bonito et al. 2010; Hall et al. 2007). Although records of white truffles in China are increasing (Fan et al. 2011a, b; Garcia-Montero et al. 2010), to date there are no records of the Magnatum clade or Gibbosum clade in China. Ten white truffles were confirmed to be in China: "sp. 2," "sp. 3," and "sp. 4" in Wang et al. (2007), *Tuber excavatum* Vittad., *Tuber latisporum* Juan Chen & P. G. Liu, *Tuber lijiangense* L. Fan & J. Z. Cao (*Tuber borchii var. sphaerosporum* sensu Juan Chen & P. G. Liu), *Tuber liui* A. S. Xu, *Tuber polyspermum* L. Fan & C. L. Hou, *Tuber sinoexcavatum* L. Fan & Yu Li, and *Tuber zhongdianense* X. Y. He et al. (Chen and Liu 2007; Chen et al. 2008; Fan et al. 2011a, b; Wang et al. 2007). Although *Tuber borchii* Vittad. or *T. puberulum*-like species have been reported from China, the occurrence of *T. borchii* and *Tuber puberulum* Berk. & Broome in China seems doubtful, as morphology and DNA sequences generally distinguish European and Asian species (Chen and Liu 2007; Fan et al. 2011a; Wang 1988; Wang et al. 2007). In some markets in southwestern China, white truffles are sold unintentionally

mixed together with commercial black truffles. It is hard to assess the commercial value of the Chinese white truffles, for all of them are represented by very rare/small collections and there is no report on the edibility. Nevertheless, according to phylogenetic analysis, *T. zhongdianense* and *T. liui*, two truffles endemic to subalpine regions in southwestern China, could be good candidates for commercialization and cultivation due to their close relationship with *T. borchii* (Chen and Liu 2007; Wang et al. 2007).

## 13.2.2 Mycorrhizal Syntheses

Following methods popularized in Europe, cultivation of truffles in China began with mycorrhizal synthesis. The first mycorrhizal synthesis in China with truffles was made by H. T. Hu, National Taiwan University, Taipei, in 1987 by using *T. formosanum* (Hu et al. 2005). Spore suspension was inoculated on a Chinese fagaceous tree *C. glauca* with a dose of  $1.2 \times 10^5$  spores/seedling. Hu et al. (2005) did not provide record when mycorrhizae began to form, but one year later, mycorrhizae were confirmed (Hu 1992; Hu et al. 2005), and the infected seedlings were then transplanted to establish a truffière.

After Hu's work, there were no more reports on Chinese truffle mycorrhizal synthesis until 2002. However, between 1997 and 2002, European researchers synthesized mycorrhizae by using truffles collected from China, either to obtain mycorrhizae for taxonomic comparison or identification or to test the ability of Chinese truffles to form mycorrhizae with exotic trees. Comandini and Pacioni (1997), Zambonelli et al. (1997), and Mabru et al. (2001) reported successful mycorrhizal synthesis between Chinese black truffles and European tree hosts. In total, mycorrhizae of *T. indicum* s.l. were produced on four European tree species: *Quercus pubescens* Willd., *Quercus cerris* L., *Pinus pinea* L., and *Corylus avellana* L., which indicates a broad host spectrum for the *T. indicum* complex.

During the period 2003–2009, researchers from southwestern China tried inoculating *T. indicum* s.l. on 16 tree species and successfully obtained mycorrhizae (Chen 2003; Geng et al. 2009; Hu et al. 2004, 2006; Lin et al. 2008). These tree species include the following: three pines (*P. armandii, Pinus massoniana* Lamb., *P. yunnanensis*), ten fagaceous trees (*C. glauca, C. mollissima, Castanopsis delavayi* Franch., *Castanopsis fargesii* Franch., *Cyclobalanopsis gilva* (Bl.) Oerst., *Cyclobalanopsis myrsinifolia* (Bl.) Orest., *Cyclobalanopsis nubium* (Hand.-Mazz.) Chun ex Q. F. Zheng, *Quercus acutissima* Carruth., *Quercus aliena* Bl., *Quercus fabri* Hance), two hazels (*Corylus* sp., *C. yunnanensis* (Franch.) A. Camus), and *Carpinus pubescens* Burk. More recently, Bonito et al. (2011) showed that *T. indicum* s.l. also established ectomycorrhizae with North American host species including pecan (*Carya illinoinensis* (Wangenh.) K. Koch) and loblolly pine (*Pinus taeda* L.). Lin et al. (2008) tried to inoculate *T. indicum* s.l. on two plants frequently occurring in the natural habitat of *T. indicum* s.l., *Coriaria nepalensis* Wall. and *Ficus tikoua* Burk., but failed to get mycorrhizae. In agreement with the European findings,

the work above confirms a very broad host spectrum for *T. indicum* s.l. Although most of these tree species have not been used for establishing truffière, the broad host spectrum suggests a feasibility of *T. indicum* cultivation in China in the future using a range of diverse host species.

Besides work on Chinese truffles, mycorrhizal synthesis between exotic truffles and Chinese trees were also tried in China. In 2002, researcher from Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou, reported mycorrhizal synthesis between T. melanosporum and a Chinese tree, Castanopsis hystrix Miq. (Chen 2002). Mycelia of T. melanosporum introduced from France were used as inoculum. Inoculum was injected into the substrate 3 days after the seedlings were transplanted. Mycorrhizae formed 26 weeks after inoculation. However, according to the morphological description, abundant tapering cystidia that measured 100 µm long and had 2-3 septa grew on the surface of mantle, which did not seem to represent mycorrhizae of T. melanosporum. Later researchers from the same institute, Gong et al. (2003), reported successful mycorrhizal synthesis between T. melanosporum and five Chinese trees: Castanopsis fissa (Champ. ex Benth.) Rehd. & Wils., C. delavayi, P. yunnanensis, P. massoniana, and Quercus variabilis Bl. The methods are similar to that of Chen (2002). All of these trees formed mycorrhizae with T. melanosporum, with infection ratio of 90–100 %. Chen (2003) tried mycorrhizal synthesis by using Italian white truffle T. magnatum Pico in Guizhou. However, it is not clear if mycorrhizae were synthesized successfully.

By using the combination of T. indicum with P. massoniana and P. armandii, Hu et al. (2006) compared inoculating methods, spore density, and seedling age and evaluated different methods of inoculation on mycorrhizal formation. They found that the best way to pretreat the seedlings is to cut the main root of seedlings before new leaves emerge and then inoculate the remaining root system. They found that winter (November) is the best season for inoculation in Guizhou. Dose of inoculum is a very practical issue in producing truffle seedlings. Hu et al. (2006) also found that as the concentration of spores increased, the number of mycorrhizae is also increased. The highest spore numbers they used are  $1.24 \times 10^{5}$ /seedling for *P. massoniana* and  $1.82 \times 10^{5}$ /seedling for *P. armandii*. This is comparable with Hu et al. (2005) for T. formosanum (1.2  $\times$  10<sup>5</sup>/seedling) but more economical than that used by Geng et al. (2009) (5  $\times$  10<sup>7</sup>/seedling) and Hu et al. (2004) for fagaceous trees  $(3.2-3.6 \times 10^6)$ /seedling). A detailed description of process was given by Geng et al. (2009), which can be summarized as follows: Aqueous spore suspension is prepared by blending chopped ascomata within water. Spore concentration is measured with a hemacytometer. Seeds are surface sterilized with H<sub>2</sub>O<sub>2</sub> and sown in sterilized perlite/vermiculite. At the age of 3 months, seedlings are transplanted in container with substrate consisting of humus/vermiculite/peat moss or humus/soil/limestone, previously steam sterilized for 3 h. Substrates are limed before planting. Inoculum is incorporated (mixed, poured, or injected) into the soil substrates either before or after planting. Pre-inoculating in soil when sowing seeds is not recommended (Hu et al. 2006). This is the most popular procedure in producing truffle seedlings in China.

## 13.2.3 Ecology

#### 13.2.3.1 Climate and Landscapes

Black truffles have been found in two regions of China: Taiwan and southwestern China. Nantou, the location in Taiwan, is characterized by a humid climate and an elevation of 1,000 m. Distribution of truffles in southwestern China is rather narrow, mainly in the adjacent localities between Sichuan and Yunnan. The most southern localities are situated south to Kunming. In southwestern China, truffle localities are characterized by rather high elevation (1,500–2,800 m) and expand across a subtropical monsoon climate zone (Liu et al. 2008; Su 2005; Tao and Liu 1990; Zhang and Wang 1990). The only fruiting plantation in mainland China is situated in central Guizhou, a mountainous place with elevation of 1,150–1,450 m, slope of 5–15 °C, and annual precipitation of 1,100 mm, essentially meeting the climatic and geographical attributes of natural truffle habitats. Most truffle localities are situated on 5–30° slopes and annual precipitation reaches ca 1,000 mm. This makes truffle hunting very hard, and truffle habitats easily undergo serious water and soil erosion when undue hunting styles are used. Actually, the situation has become quite serious during the past 15 years.

#### 13.2.3.2 Soil

Shortly after T. sinense (= T. indicum s.l.) was described from China, researchers from China started to investigate the ecological traits of Chinese truffles, including soils. All of these investigations focused on T. indicum s.l. and in Sichuan and Yunnan, the only two provinces in mainland China where wild black truffles have been found. Most investigations found that the black truffles favor purple soils, which are calcareous and have a very broad pH range (5.5-8.5) (Chen et al. 1998; Su 2005; Yang et al. 2000; Zhang and Wang 1990). Tao and Liu (1990) and Lin et al. (2008) reported that black truffles also grow in yellow soils and acid red soils, respectively. Soil attributes are very rarely documented, except that Chen et al. (1998) provided some reference data: organic matter 1.43–5.32 (%), total N 0.085–0.375 (%), available P 5.8–49.6 mg/kg, and available K 149–388 mg/kg. Unfortunately, up to now, consistency of Ca<sup>2+</sup> and Mg<sup>2+</sup>, which is the key attributes for truffle soil, has not been well documented. However, based on the distribution of truffle and parent rock, it is reasonable to suppose high levels of  $Ca^{2+}$  in truffle soils. An interesting experience is that in our field investigations, truffles are often found near quarries, which suggests rather rich calcareous matter in the soil.

Soils in the first fruiting truffière in China were analyzed by Hu et al. (2005). Since they had been limed before transplantation, the soils were characterized by high pH values ( $6.3 \pm 1.1$ ) and levels of Ca<sup>2+</sup> ( $91 \pm 73$  ppm). The first fruiting truffière in mainland China were in yellow soils with clay loam or sandy clay loam and an original pH of 5.5–6.0 (Longli, Guizhou) (Hu et al. 2010). Liming with

quicklime or CaCO<sub>3</sub>/MgCO<sub>3</sub> may aid fruiting of truffles in the present, but the potential for sustainable long-term yields has not been assessed.

### 13.2.3.3 Host Plants

Black truffles can be found both in pure stands of *P. yunnanensis* and *P. armandii* and mixed forests with fagaceous trees. Three coniferous trees are found to be associated with *T. indicum* s.l.: *P. yunnanensis*, *P. armandii*, and *Keteleeria evelyniana* Mast. in natural habitats (Su 2005; Zhang and Wang 1990). Mycorrhizal synthesis confirmed the symbiotic relationships between *T. indicum* s.l. and the two pines (Chen 2003; Geng et al. 2009; Hu et al. 2004; Lin et al. 2008). *Pinus yunnanensis* is the most common pine in central and north Yunnan and southern Sichuan. *Pinus armandii* is a five-needled pine, distributed through north and central China to central and north Yunnan. *Keteleeria evelyniana* is a coniferous tree endemic to Yunnan. The wide distribution of these host trees implies a potential broader distribution of black truffles in China.

Besides the confirmed host trees, in natural habitats of black truffles, the following trees and shrubs also frequently occur: *Quercus acutissima* Carruth., *Quercus franchetii* Skan, *C. mollissima*, *C. delavayi*, *Coriaria sinica* Maxim., *Vaccinium bracteatum* Thunb., *Phyllanthus emblica* L., and *F. tikoua* (Chen et al. 1998; Su 2005; Tao and Liu 1990; Zhang and Wang 1990; our own investigation). Among them, some fagaceous trees, such as *Q. acutissima*, *C. delavayi*, and *C. fargesii* can form mycorrhizae with *T. indicum* s.l. in artificial conditions (Hu et al. 2004; Liu et al. 2008). Since black truffles often grow in mixed woods, these trees may also be mycorrhizal partners in the wild.

The frequent occurrence of *Alnus* spp. and *Coriaria* spp. in the natural habitats of black truffles is interesting and informative. Recent investigations on bacterial communities in ascomata of *T. magnatum* detected nitrogen-fixing bacteria and nitrogen-fixing activates within truffle (Barbieri et al. 2010; Chap. 8). As we have known, some trees of *Coriaria* and *Alnus* also have nitrogen-fixing bacteria inhabiting in their root systems. It is possible that bacteria shared by the ascomata of black truffles and trees of *Coriaria* and *Alnus* play an important role in the life cycle of both the fungi and the plants. Future research is needed on the application of helper bacteria to mycorrhization and cultivation of truffles.

## **13.3** Advantages, Challenges, and Future

The successful cultivation of truffles in China, although still at a limited scale, demonstrates the possibility for cultivating truffles in mountainous regions of China. During the past almost 25 years, seven institutions have conducted truffle cultivation trials and have a number of truffle plantations in the country. It is hopeful that some of these plantations will see the first fruiting in the coming 3–5 years.

Truffle seedling production is well developed, and some basic data on host trees, soil, and distribution have been obtained. The broad host spectrum of Chinese black truffles could facilitate the selection of trees for cultivation and help to establish diverse truffle plantations. In southwestern China, especially in its southern and eastern parts where typical karst landscapes are well developed (southeastern Yunnan, Guizhou, northern Guangxi), soil between rocks is poor for crops. However, these soils are rich in Ca<sup>2+</sup> and may be ideal sites for truffle cultivation. The vast mountainous regions in central and southern China will make truffle cultivation prosperous. Cheaper land and labor will make Chinese truffle products more competitive in international markets if standards and quality are maintained.

At the same time, China faces big challenges in truffle cultivation. Although initial successes have been made, the sustainability of truffle harvesting practices and quantities remain open questions. There is no continuous record on the productivity of truffles for the first truffière in Taiwan. We noticed that in the first truffière in mainland China, quicklime was used to increase the pH of soil. Since efficiency of quicklime could be exhausted out in short time, the stability of annual yield may not be sustained. To our knowledge, most plantations in southwestern China are close to natural forests with ectomycorrhizal hosts or are situated on land that used to be natural forests. This does not meet the standard for truffle cultivation, which recommends that fields are free of roots from other ectomycorrhizal hosts. However, due to the large population in China and shortage of arable land, it is hard to find optimal truffle sites in mountainous regions in China (or even plains).

Most truffle plantations in China cultivate *T. indicum* s.l. at a slim profit margin. China does not have a strong truffle culture, and truffles in China have a small market. The major markets for Chinese truffles are still in Europe, supplemented by Japanese and American markets. In Europe, Asian black truffles are regarded as inferior in taste and flavor than *T. melanosporum* and even as fraud (Mabru et al. 2001; Paolocci et al. 1997). The price of Chinese truffles exported to Europe varies with season and supply but normally within the range of \$50–100/kg. Compared with *T. melanosporum* and even *T. aestivum/uncinatum*, whose prices are more than ten times this, the profit of cultivating indigenous truffles in China is rather low. According to Lefevre and Hall (2001), annual yields of 15–20 kg/ha are considered good. Most Chinese plantations are still unproductive and reliable data on yields are lacking, but according to Hu et al. (2005), the yield of *T. formosanum* is 10 kg/ 0.6 ha. Although this yield seems reasonable, because of the lower value of this species, this yield brings 1/10 the profit that a plantation of *T. melanosporum* with the same yield.

There are two ways to improve the profitability of truffle cultivation in China. First, truffières should be established using economic species of trees. In south-western China, two economic trees that are able to produce black truffles are P. *armandii* and C. *mollissima*. Both of these tree species are used by the local people for nut production; therefore, they offer an effective supplement for the value of truffles. A second approach for improving the profitability of truffle cultivation in China is to cultivate European truffle species such as T. *melanosporum* or T. *aestivum*. Some experiments have shown that some Chinese tree species can



Fig. 13.1 Immature Tuber indicum hunted by digging the soil

become well colonized by *T. melanosporum* (Gong et al. 2003). One plantation in Guizhou has already demonstrated the possibility to grow European truffles in China, though yields are still small (Gong 2009). The cultivation of European truffles in New Zealand and Australia demonstrate the possibility for developing a truffle industry with these species outside their native localities.

From a comprehensive view, truffle cultivation in China is not only a matter of money but also a matter of environmental protection. It should be considered as a huge project with multiple functions. In mountainous China, plant crops are not allowed to be planted on slopes  $>30^\circ$ , but forestation is allowed. The government compensates the farmers for giving up the lands and provides seedlings to the farmers for reforestation. All truffle seedlings now produced in China use indigenous trees, well adapted to the local soil and climate. With help from mycorrhizae, these seedlings can tolerate and adapt to stressful habitats. In such cases, planting truffle-inoculated seedlings could provide multiple functions: harvesting truffles and helping to recover the vegetation. The "two-in-one" function of planting truffle seedlings will make the reforestation in mountainous regions easier to accept. The ecological significance of truffle cultivation in China provides additional value to such an effort.

Another factor, which may not be the most technical but will be the final step to guarantee success of truffle cultivation in China, is the use of truffle dogs for harvesting. Black truffles ripen from December to March in China. However, hunting truffles begins early in August. Local people usually use pickaxe to dig truffle land, and hunting truffles in China is laborious and destructive (Fig. 13.1). According to fragmentary reports of media, yield of black truffle in Panzhihua, "home of truffles in China," has decreased to 30–40 tons from 200 tons in 1990s. Even worse, since harvesting is too early to get mature products, the quality of truffles is seriously compromised. Tan and Fu (2003) investigated the quality of ascomata sold in three local markets in southern Sichuan and found that at most half

of the ascomata were mature and in good shape. Because immature truffles lack the aroma and flavor of mature specimen, the reputation of Chinese truffles suffers by such practices. Without truffle dogs, it is hard to imagine how truffle plantations can provide products of high quality, how they can be maintained, and how truffle yields can be sustained. It is reported that in Panzhihua, Sichuan, under the direction of researchers from IPLA, trials of training truffle dogs have been successful (Lin et al. 2008). Some researchers in Yunnan are also starting projects to train truffle dogs. It may take time for the local farmers to accept and adopt the use of truffle dogs, but good demonstrations on the value of truffle dogs for increasing truffle harvests, quality, truffle value, and profit are likely to facilitate their adoption.

As a country with a short history of truffle cultivation, China still has a long way to go. Truffle soil, a key factor in truffle cultivation, is poorly documented. There is still limited data available on feasibility or site selection. High-quality truffleinoculated seedlings, a critical component in successful truffle cultivation, have not been qualified by strict commercial standards. To improve the profit of truffle cultivation in China, further mycorrhizal syntheses between European culinary truffles and Chinese trees need to be conducted. When establishing plantations, experimental designs should test the effects of limestone amount, limestone composition, fertilization, irrigation, planting density, and mulching. Experience on weed control, tilling, and pruning in truffle orchards is also lacking in China. Adopting best management practices from Europe and other regions and popularizing the use of truffle dogs in China may take time or even financial support. Nevertheless, a growing number of farmers, companies, and research institutions are showing enthusiasm for this new industry in China.

## 13.4 Conclusions

Although China has a much shorter history of scientific research on truffles, the country has seen the successful cultivation of T. indicum s.l. and T. melanosporum. At least ten plantations have been established in the country. Private and public enthusiasm for truffle cultivation is increasing. A series of relevant investigations on taxonomy, ecology, and mycorrhizal synthesis on black truffles have provided important data on their cultivation. Chinese black truffles T. indicum s.l. have a broad host spectrum and are adapted to diverse soils. Mountainous regions in southern China have suitable climate, soil, and host trees for truffle cultivation. Truffle cultivation in China should be regarded as a project with economic and ecological functions. Broad area, diverse hosts, low cost, and multiple functions are advantages for developing truffle cultivation in China. However, in order to make the industry sustainable and profitable, it is urgent to set strict standards and qualifications for seedling production, plantation establishment, and harvesting methods. Time-consuming research needs to be conducted to improve the quality of plantations in China. Truffle cultivation in China needs better integration between individual farmers, research institutions, and the government.

# References

- Anonymous Reporter (2009) Project of cultivation of *T. melanosporum*. Hunan For Sci Technol 36(2):F0004
- Barbieri E, Ceccaroli P, Saltarelli R, Guidi C, Potenza L, Basaglia M, Fontana F, Baldan E, Casella S, Ryahi O, Zambonelli A, Stocchi V (2010) New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*. Fungal Biol 114:936–942. doi:10.1016/j. funbio.2010.09.001
- Bonito G, Trappe JM, Rawlinson P, Vilgalys R (2010) Improved resolution of major clades within *Tuber* and taxonomy of species within the *Tuber gibbosum* complex. Mycologia 102 (5):1042–1057. doi:10.3852/09-213
- Bonito G, Trappe JM, Donovan S, Vilgalys R (2011) The Asian black truffle *Tuber indicum* can form ectomycorrhizas with North American host plants and complete its life cycle in non-native soils. Fungal Ecol 4(1):83–93. doi:10.1016/j.funeco.2010.08.003
- Chen YL (2002) Mycorrhizal formation of Tuber melanosporum. Edible Fungi China 21(5):15-17
- Chen BT (2003) Report on inoculation and infection of truffles and establishment of truffle plantation. Guizhou For Sci Technol 31(4):10–14
- Chen J, Liu PG (2007) *Tuber latisporum* sp. nov. and related taxa, based on morphology and DNA sequence data. Mycologia 99(3):475–481. doi:doi:10.3852/mycologia.99.3.475
- Chen J, Liu PG (2011) Delimitation of *Tuber pseudohimalayense* and *T. pseudoexcavatum* based on morphological and molecular data. Cryptogamie Mycol 32(1):83–93
- Chen HQ, Liu HY, Li ZP (1998) A primary study on ecological property of truffle. Acta Edulis Fungi 5(1):39–44
- Chen J, Liu PG, Wang Y (2005) Notes on *Tuber aestivum* (Tuberaceae, Ascomycota) from China. Acta Bot Yunnan 27(4):385–389
- Chen J, Wang Y, Liu PG (2008) Two new records of *Tuber* (Pezizomycetes, Pezizales) from China. Mycotaxon 104:65–71
- Chen J, Guo SX, Liu PG (2011) Species recognition and cryptic species in the *Tuber indicum* complex. PLoS One 6(1):e14625. doi:10.1371/journal.pone.0014625
- Comandini O, Pacioni G (1997) Mycorrhizae of Asian black truffles. *Tuber himalayense* and *T. indicum.* Mycotaxon 63:77–86
- Fan L, Cao JZ, Liu YY, Liu Y (2011a) Two new species of *Tuber* from China. Mycotaxon 116:349–354. doi:10.5248/116.349
- Fan L, Hou CL, Cao JZ (2011b) *Tuber sinoalbidum* and *T*. polyspermum—new species from China. Mycotaxon 118:403–410. doi:10.5248/118.403
- García-Montero LG, Di Massimo G, Manjón JL, García-Abril A (2008) New data on ectomycorrhizae and soils of the Chinese truffles *Tuber pseudoexcavatum* and *Tuber indicum*, and their impact on truffle cultivation. Mycorrhiza 19(1):7–14. doi:doi:10.1007/s00572-008-0198-x
- Garcia-Montero LG, Diaz P, Di Massimo G, Garcia-Abril A (2010) A review of research on Chinese *Tuber* species. Mycol Prog 9(3):315–335. doi:10.1007/s11557-009-0647-8
- Geng LY, Wang XH, Yu FQ, Deng XJ, Tian XF, Shi XF, Xie XD, Liu PG, Shen YY (2009) Mycorrhizal synthesis of Tuber indicum with two indigenous hosts, *Castanea mollissima* and *Pinus armandii*. Mycorrhiza 19:461–467. doi:10.1007/s00572-009-0247-0
- Gong MQ (2009) The first success of truffle cultivation in China. Edible Fungi China 28(3):15
- Gong MQ, Chen Y, Wang FZ (2003) A study of mycorrhizal synthesis effect of *Tuber melanosporum* on seedlings. For Res 16(1):52–57
- Hall IR, Brown G, Zambonelli A (2007) Taming the truffle. The history lore and science of the ultimate mushroom. Timber, Portland, OR
- Hu HT (1992) *Tuber formosanum* sp. nov. and its mycorrhizal associations. Q J Exp For NTU 6(4):79–86
- Hu BF, Zhu ZR, Yuan XM, Yi XY, Zhang XM, Wang L, Fang S, Yang S, Liao WB (2004) Mycorrhizal synthesis of *Tuber indicum* and its effects on promoting seedling growth and preventing diseases. Guizhou For Sci Technol 32(2):19–24

- Hu HT, Wang Y, Hu BY (2005) Cultivation of *Tuber formosanum* on limed soil in Taiwan. NZ J Crop Hortic Sci 33:363–366. doi:10.1080/01140671.2005.9514371
- Hu BF, Yin XY, Zhu ZR, Yuan XM, Yang AM, Jin TX, Hu G, Liao WB, Yang P (2006) Study on the inoculation technology of *Tuber* sp. for the mycorrhizal seedlings. Guizhou For Sci Technol 34(2):15–18, 26
- Hu BF, Yuan XM, Yu JY, Wu YK (2010) First successful cultivation of *Tuber indicum* in Guizhou. For Byproduct Specialty China 2:F0003
- Huang JY, Hu HT, Shen WC (2009) Phylogenetic study of two truffles, *T. formosanum* and *Tuber furfuraceum*, identified from Taiwan. FEMS Microbiol Lett 294(2):157–171. doi:doi: 10.1111/j.1574-6968.2009.01571.x
- Lin Q, Han D, Liu CY (2008) Natural resources of truffles and analysis on the industry development in western Panzhihua. Panzhihua SciTech Inf 33(1):36–41
- Lefevre CK, Hall IR (2001) The status of truffle cultivation: A global perspective. Acta Hort 556:513–520
- Liu B (1985) New species and new records of hypogeous fungi from China (1). Acta Mycol Sinica 4:84–89
- Liu CY, Tang P, Lan H, Han D, Lin Q, Jiang Q, Sun KY (2008) Study on impact of *Tuber indicum* mycorrhizae on the growth of tree saplings. J SW For Coll 28(6):21–24
- Mabru D, Dupré C, Douet JP, Leroy P, Ravel C, Ricard JM, Médina B, Castroviejo M, Chevalier G (2001) Rapid molecular typing method for the reliable detection of Asiatic black truffle (*Tuber indicum*) in commercialized products: fruiting bodies and mycorrhizal seedlings. Mycorrhiza 11:89–94. doi:10.1007/s005720100103
- Manjón JL, Garcia-Montero LG, Alarado P, Moreno G, Di Massimo G (2009) Tuber pseudoexcavatum versus T. Pseudohimalayense – new data on the molecular taxonomy and mycorrhizae of Chinese truffles. Mycotaxon 110:399–412. doi:10.5248/110.399
- Paolocci F, Rubini A, Granetti B, Arcioni S (1997) Typing *Tuber melanosporum* and Chinese black truffles by molecular markers. FEMS Microbiol Lett 153:255–260. doi:10.1016/S0378-1097(97)00226-7
- Roux C, Séjalon-Delmas N, Martins M, Parguey-Leduc A, Dargent R, Guillaume Bécard G (1999) Phylogenetic relationships between European and Chinese truffles based on parsimony and distance analyses of ITS sequences. FEMS Microbiol Lett 180:147–155. doi:10.1016/S0378-1097(99)00474-7
- Song MS, Cao JZ, Yao YJ (2005) Occurrence of Tuber aestivum in China. Mycotaxon 91:75-80
- Su KM (2005) Ecological investigation on truffles in Yongren County, Chuxiong Pref., Yunnan. Edible Fungi China 24(4):12–13
- Tan ZM, Fu SC (2003) Issues on truffle harvesting and trade in China. Edible Fungi China 22(4):3-5
- Tao K, Liu B (1990) Ecology and nutritive value of *Tuber sinense*. J Shanxi Univ Nat Sci Ed 13(3):319–321
- Tao K, Liu B, Zhang DC (1989) A new species of the genus *Tuber* in China. J Shanxi Univ Nat Sci Ed 12(2):215–218
- Wang Y (1988) First report of study on *Tuber* species from China. In: Atti del II Congresso Internazionale sul Tartufo, Spoleto, 24–27 Nov 1988, pp 45–50
- Wang YJ, Tan ZM, Zhang DC, Murat C, Jeandroz S, Le Tacon F (2006) Phylogenetic and populational study of the *Tuber indicum* complex. Mycol Res 110:1034–1045. doi:10.1016/j. mycres.2006.06.013
- Wang YJ, Tan ZM, Murat C, Jeandroz S, Le Tacon F (2007) Molecular taxonomy of Chinese truffles belonging to the *Tuber rufum* and *Tuber puberulum* groups. Fungal Divers 24:301–328
- Yang FY, Yang C, Zhang XL (2000) The investigation of "Chinese Root Mushroom" in Liangshan Prefecture and its development. J Xichang Agric Coll 14(1):51–55
- Zambonelli A, Tibiletti E, Pisi A (1997) Caratterizzazione anatomo-morfologica delle micorrize di Tuber indicum Cooke & Massee su Pinus pinea L. e Quercus cerris L. Micol Ital 28(1):29–36

- Zambonelli A, Iotti M, Piattoni F (2012) Chinese *Tuber aestivum* sensu lato in Europe. Open Mycol J 6:22–26
- Zang M, Pu CX, Wu JM, Chen QF, Liu B (1992) Confirmation of the distribution of *T. indicum* in China. Edible Fungi China 11(3):19–39
- Zhang DC, Wang Y (1990) Study on Chinese truffles and its ecology. Edible Fungi China 9(2):25-27
- Zhang LF, Yang ZL, Song DS (2005) A phylogenetic study of commercial Chinese truffles and their allies: taxonomic implications. FEMS Microbiol Lett 245:85–92. doi:10.1016/j. femsle.2005.02.028