



## Burgundy Black Truffle Cultivation in an Agroforestry Practice

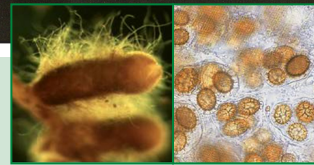
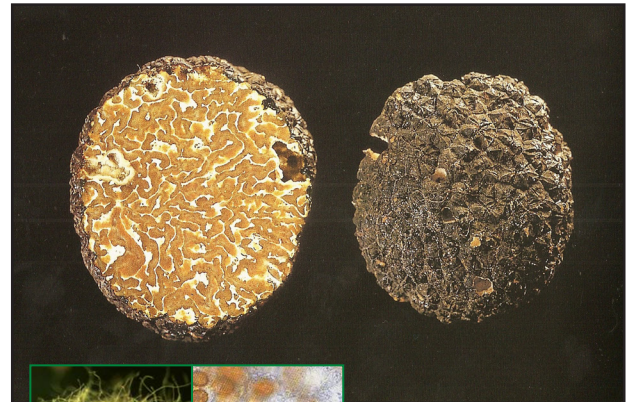
by **Johann Bruhn, Ph.D.**, Research Associate Professor, Division of Plant Sciences, University of Missouri, & **Michelle Hall**, Senior Information Specialist, The Center for Agroforestry, University of Missouri

The two best candidate species for truffle cultivation in the south-central U.S. are the Burgundy truffle (*Tuber aestivum* Vitt., syn. *T. uncinatum* Ch., Fig. 1a) and the Périgord black truffle (*T. melanosporum* Vitt., Fig. 1b). These common names (Burgundy truffle and Périgord black truffle) are derived from the names of two of the many regions in France where they are famous. Though native to Europe, there is great interest in expanding the cultivated range of both of these species.

Each of these two truffle species has unique life cycle features and characteristic habitat requirements. Although the retail price of Périgord black truffles is roughly twice that of Burgundy truffles, Périgord truffles mature during the winter and are destroyed if they thaw in the ground after having frozen solid. In contrast, Burgundy truffles mature mainly in late autumn, before danger of the soil freezing.

In addition, the Burgundy truffle fungus can produce higher yields per acre, perhaps because it typically grows in the shade of denser stands of trees. Denser stands of trees also serve to more effectively filter and cleanse groundwater (for example, Allen et al. 2004).

The recently discovered nitrogen fixation activities of truffles (see "The Burgundy truffle as a ectomycorrhizal fungus," below) places them in the company of legume nodules as agents of soil improvement (Barbieri et al. 2010).



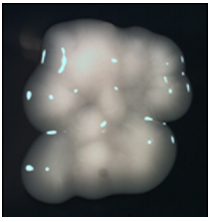
Burgundy black truffle, above. The white veining is composed of sterile tissue supporting

the production of colorful spores. Mature spores are brown. Mycorrhizal root tips formed by the Burgundy truffle are easily recognized by their ornamentation and cell structure (left inset). Ascospores of the Burgundy truffle are caramel colored with a net-like system of ridges (right inset).

### Table of Contents

The Burgundy Truffle as a Mycorrhizal Fungus, pg. 2
Why the Burgundy Truffle? pg. 4
Choosing a Tree Species, pg. 5
Characteristics of a Good Plantation Site, pg. 6
Plantation Site Preparation, pg. 10
Plantation Establishment, pg. 11
Plantation Maintenance, pg. 13
Harvesting, pg. 14
Cooking with Truffles, pg. 16
Additional Resources, pg. 17





*This strain of Bradyrhizobium is one of many that we have isolated into pure culture from within Burgundy truffles. Bradyrhizobium species fix atmospheric nitrogen in legume nodules, but are also believed to fix nitrogen inside developing truffles. As a result, Burgundy truffle cultivation may improve soil quality in a manner similar to legume cultivation.*

The Burgundy truffle fungus also appears to grow and fruit more competitively over a wider range of soil conditions than the Périgord truffle fungus. Where appropriate conditions persist, the Burgundy truffle fungus appears capable of sustained fruiting over centuries (Weden et al. 2004b).

Significantly, most of what we know about the biology and cultivation of European black truffles is based on research and experience in their native ranges in Europe, and much of the scientific literature concerning the biology and cultivation of the Burgundy truffle has been published in European languages (for example, Chevalier and Frochet 1997, Olivier et al. 2002, Wedén 2008). Nevertheless, work in New Zealand (Hall et al. 2007) has demonstrated that the Périgord black truffle can be successfully cultivated across a wider range of soil conditions than constitute its native European range, and this may prove true for the Burgundy truffle as well.

English language reports of research into the biology, ecology and cultivation of the Burgundy truffle (for example, Delmas 1978; Chevalier et al. 2001; Weden et al. 2004a, 2004b and 2009; Zambonelli et al. 2005; Hall et al 2007; Pruett et al. 2008a, 2008b, 2009; Bruhn et al. 2009; Wehrlen 2008, 2009; Wehrlen et al. 2009) have helped support research globally. In the face of limited research support, the spirit of collegiality among scientists around the world reflects the universal desire to jointly advance these topics as quickly as possible.

Despite all that is known about truffle biology and cultivation, efforts to cultivate these exquisite mushrooms meet with variable success, often for reasons not yet understood. We might conclude that successful trufficulture today is largely based on science, with significant room for inspiration and luck.

For all of these reasons, the Burgundy truffle appears better suited than the Périgord black

truffle as a sustainable agroforestry specialty crop under Missouri conditions. The Burgundy truffle is probably the most widely eaten truffle species in Europe (RiOUSset et al. 2001). The biology, ecology, cultivation and use of the Burgundy truffle are the subjects of this publication.

Before discussing cultivation of the Burgundy truffle, we need to understand (to the best of current knowledge) the biology and ecology of this remarkable fungus.

### *Truffle cultivation is a perplexing endeavor for several key reasons, including:*

- 1) the complex mycorrhizal nature of truffle fungi;
- 2) the long period between truffle orchard establishment and truffle fruiting; and
- 3) the fact that Burgundy truffle “mushrooms” typically form submerged in the soil.

## The Burgundy Truffle as a Mycorrhizal Fungus

Truffle fungi depend for their existence on the successful establishment of a mutually beneficial nutritional relationship with the root systems of receptive tree species (so-called “host” trees). This sort of mutualistic symbiosis between root-colonizing truffle fungi and receptive tree species is representative of one form of “mycorrhizal” relationship.

*The word mycorrhiza (plural, mycorrhizas; adjective, mycorrhizal) comes from two Greek words that translate roughly as “fungus root.”*

In simplest terms, a truffle fungus modifies root tip anatomy of receptive plants to facilitate the exchange of sugar produced by the plant (through photosynthesis) for minerals and water gathered by the fungus from the soil. Nearly all land plant species require some form of mycorrhizal relationship to survive (Smith and Read 1997). Due to its finely branched structure, the fungal weft, comprising myriad microscopic filaments, is able to forage among soil particles far more efficiently than the fine roots of the plant alone.

Most tree species associate with fungi that produce either “ectomycorrhizas” or “arbuscular mycorrhizas.” The fungi that produce ecto- vs. arbuscular mycorrhizas are quite unrelated. Truffle fungi produce ectomycorrhizas. “Ecto” is the Greek prefix meaning “outside,” referring to the fact that ectomycorrhizal fungi affect the outward appearance of colonized root tips. “Arbuscular” is the Greek adjective meaning “tree-shaped,” referring to the tree-shaped “arbuscules” produced by the fungus within fine root cortex cells. Arbuscular fungi do not noticeably modify root tip appearance. It’s important to know what kind of mycorrhizas different tree species form because arbuscular mycorrhizal fungi won’t replace an ectomycorrhizal fungus. Thus, truffle-colonized (ectomycorrhizal) oak seedlings can be interplanted among (arbuscular mycorrhizal) apple trees to take advantage of the shade provided by the apple trees. Table 1 provides lists of some common ectomycorrhizal and arbuscular mycorrhizal tree species.

Fortunately, the ectomycorrhizas produced by the Burgundy truffle fungus are quite distinctive and can generally be discerned under the dissecting and/or compound microscope from those produced by other ectomycorrhizal fungi (Müller et al. 1996; Chevalier and Frochot 1997). Nevertheless, nurserymen, consultants and plantation owners alike should have the mycorrhizal condition of their greenhouse seedlings and plantation trees evaluated by an impartial laboratory. These confirmations should consist at least of a systematic microscopic evaluation. Ideally, this evaluation should be accompanied by extraction and identification of fungal DNA from samples of tentatively identified truffle mycorrhizas (for example, Iotti and Zambonelli 2006). This process is increasingly affordable, and provides important assurances to all parties involved at every stage of truffle cultivation.

Systems for the independent certification of greenhouse seedling colonization are widely used in France (Chevalier and Grente 1978), Italy (Bencivenga et al. 1995), and Spain (Fischer and Colinas 1996). Seedlings from greenhouse production batches that have been certified to be well colonized by the intended truffle species are sold bearing tags affirming their certification and identifying the certifying agency. Systematic independent certification has yet to be established elsewhere. Caveat emptor... Let the buyer beware!

Table 1. Some tree species known to associate with arbuscular mycorrhizal fungi and/or ectomycorrhizal fungi.

Common name	Scientific name	Arbusc.	Ecto.
Apple	<i>Malus</i>	A	
Ash	<i>Fraxinus</i>	A	
Aspen, cottonwood, poplar*	<i>Populus</i>	A	E
Bamboo	<i>Bamusa</i>	A	
Beech	<i>Fagus</i>		E
Black locust	<i>Robinia</i>	A	
Box elder	<i>Acer negundo</i>	A	
Buckeye, horse chestnut	<i>Aesculus</i>	A	
Catalpa	<i>Catalpa</i>	A	
Cherry, peach, plum*	<i>Prunus</i>	A	E
Chestnut	<i>Castanea</i>		E
Dogwood	<i>Cornus</i>	A	
Ginkgo	<i>Ginkgo</i>	A	
Grape	<i>Vitis</i>	A	
Hackberry	<i>Celtis</i>	A	
Hazelnut	<i>Corylus</i>		E
Hickory, pecan	<i>Carya</i>		E
Holly	<i>Ilex</i>	A	
Hornbeam	<i>Carpinus</i>		E
Juniper	<i>Juniperus</i>	A	
Linden	<i>Tilia</i>		E
Magnolia	<i>Magnolia</i>	A	
Maple	<i>Acer</i>	A	
Mulberry	<i>Morus</i>	A	
Oak	<i>Quercus</i>		E
Persimmon	<i>Diospyros</i>	A	
Pine	<i>Pinus</i>		E
Redbud	<i>Cercis</i>		E
Tulip	<i>Liriodendron</i>	A	
Viburnum	<i>Viburnum</i>	A	
Walnut	<i>Juglans</i>		E
Willow*	<i>Salix</i>	A	E



An Agritruffe oak seedling certified by the French INRA to be colonized by the Burgundy truffle. (Inset) Examples of French and Spanish tags used to label seedlings certified to be colonized by truffle fungi.

### Truffle life cycles comprise two independent phases:

- 1) The nutritional relationship between the truffle fungus, its partner tree, and associated microbes; and
- 2) The sexual reproduction of the fungus in the form of truffle mushrooms.

This issue is difficult to study because Burgundy truffle production commonly lags planting of mycorrhizal tree seedlings by six to 10 years (Chevalier et al. 2001, Wedén et al. 2009). Full production may be reached by orchard age 12-15 years, and is commonly associated with the establishment of shade and a litter layer.

Finally, when (and if) Burgundy truffles do develop in a plantation, they generally form completely below the soil surface. As a result, effective harvest and evaluation of production requires the services of a well-trained dog to find them as they mature.

So, to summarize, if you do everything right (intentionally and with some good luck), your truffle orchard will eventually yield tens of pounds of truffles per acre for years, but if you make any error you won't even know it for 10 years, but you will have a very pleasant stand of lovely trees ! This guide is designed to provide a general knowledge of truffle biology and the state-of-the-art of Burgundy truffle cultivation. We have taken care to draw attention to the limits of our understanding, so that the potential trufficulteur (truffle grower) can balance the potential for success with the risks of failure, based on current knowledge and its apparent gaps.

## Why the Burgundy Truffle?

The success of a truffle cultivation effort depends on the matching of a truffle species with a receptive tree species on a mutually conducive site. Although the Burgundy and Périgord black truffle species share common features, they differ markedly in a number of important ways that influence their productivity under management.

First, the Burgundy truffle matures mainly from September through January in Burgundy, France, and from late August through early December on Gotland, Sweden (Chevalier and Frochot 1997, Wedén et al. 2004b). In contrast, the Périgord truffle fruits

To make things more “interesting,” it is clear now that mycorrhizal fungi (truffle fungi included) associate with a wide variety of bacteria that influence mycorrhiza formation and function (for example, Frey-Klett et al. 2007, Bonfante and Anca 2009). Quite recently, it has been shown that truffle fruit bodies also host large and diverse bacterial communities (Barbieri et al. 2005, 2007), that include nitrogen-fixing bacteria (Cerigini et al. 2008, Barbieri et al. 2010) closely related to those that nodulate the root systems of legumes. The roles of bacteria present in large numbers in Burgundy truffle mycorrhizas and fruit-bodies are the subject of ongoing research (Bruhn, Emerich, Wedén, and Backlund, in preparation).

The introduction of selected bacterial strains into truffle seedling production systems is an exciting prospect (for example, Thrall et al. 2005). One of the difficulties in resolving the requirements for truffle cultivation is the potential disconnect between the successful establishment of a mycorrhizal root system and its subsequent production of truffle mushrooms. In other words, a successful mycorrhizal relationship is essential to successful fruiting but may not guarantee it. It remains to be determined how similar are the bacterial communities associated with mycorrhiza formation and function, and truffle mushroom initiation and development.

during the winter, maturing between early December and mid-March in Europe (Olivier et al. 2002). This difference is supremely important, because when truffles freeze in the ground, they rot upon thawing and are unmarketable (Riousset et al. 2001, Chevalier and Frochot 1997). Thus, the Périgord black truffle is poorly suited to locations where the soil routinely freezes solid for more than a couple of days at a time during winter. For this reason, we recommend the Burgundy truffle for cultivation throughout Missouri.

Second, in locations where the soil does not characteristically freeze during the winter, the choice of Périgord or Burgundy truffle is more a matter of economics and preference. With its stronger fragrance and flavor, top-quality Périgord black truffles have sold in recent years for approximately \$900/lb. in the U.S., roughly twice the price of the Burgundy truffle. Both species of truffle are approximately twice as expensive in the U.S. as in Europe, due to their greater availability in Europe. Most Périgord black and Burgundy truffles currently sold in the U.S. are imported from Europe, with some very notable exceptions (<http://www.tennesseetruffle.com/>). Price also varies substantially with individual truffle size and quality. In these early times, both Périgord black and Burgundy truffles are in great demand at exclusive restaurants engaging in haute cuisine. Knowledgeable Missourians appear more willing to experiment with the purchase of a Burgundy truffle, due to the price differential.

Third, yields of the Burgundy truffle can be greater than those of the Périgord truffle, in part due to the greater planting density recommended for Burgundy truffle orchards (see “Plant Density”, under “Orchard Establishment”, below). Further, the Burgundy truffle fungus is generally considered to be more aggressive than the Périgord truffle in competing with coexisting native mycorrhizal fungi, so plantations established with the Burgundy truffle can have a longer productive life. Finally, the greater tree density in Burgundy truffle orchards creates a much more forested environment, which may be seen as a value in itself, improving groundwater filtration, wildlife habitat, and nitrogen fixation associated with the truffle fungus.

## Choosing a Tree Species

The Burgundy truffle fungus is capable of associating with a wide range of tree species, but experience shows that truffle production is greater in partnership

Table 2. A list of tree species known to associate with the Burgundy truffle (Chevalier et al. 2001).

Tree species	Common name
<i>Carpinus betulus</i>	European hornbeam
<i>Cedrus atlantica</i>	cedar
<i>Corylus avellana</i>	common hazelnut
<i>C. colurna</i>	Turkish hazelnut
<i>Ostrya carpinifolia</i>	hop hornbeam
<i>Q. petraea</i>	sessile oak
<i>Q. pubescens</i>	pubescent oak
<i>Q. robur</i>	common oak
<i>Pinus nigra</i> ssp. <i>austriaca</i>	Austrian black pine

with some tree species than with others. Table 2 presents a list of tree species known to associate well with the Burgundy truffle (Chevalier et al. 2001). First, an appropriate tree species needs to be receptive to fine-root colonization by the Burgundy truffle fungus both in the greenhouse and at the plantation site. So, it also seems important to use a tree species that is preferentially colonized by the Burgundy truffle fungus after outplanting in the field. For example, the Burgundy truffle is very productive with the European hazelnut (*Corylus avellana* L.), yet *C. avellana* seems to be quite receptive to a wide variety of competing ectomycorrhizal fungi as well.

It is also important to select a tree species that will grow well under existing climate and soil conditions at the plantation site. For example, the common oak (*Quercus robur* L.) is one of the principal tree species (along with *C. avellana*) associated with the Burgundy truffle on Gotland. Yet seed sources (provenances) of *Q. robur* vary in their cold hardiness and in their tolerance of the high soil pH levels required (above 7.0). These environmental sensitivities need to be considered when selecting seed sources.

It's tempting to want to select a proven tree species from the native range of the Burgundy truffle, but native North American tree species should not be automatically ruled out. For example, native tree species resistant to local diseases and pests may prove to be good Burgundy truffle associates compared to related native European species. Examples of diseases which require consideration in the U.S. include: northern filbert blight (caused by the fungus *Anisogramma anomala*), which can be especially severe on European hazelnut; chestnut

blight (caused by the fungus *Endothia parasitica*) on oaks and chinquapin; and powdery mildew of common oak (caused by several fungi, including *Microsphaera alphitoides*). For example, we have found that the hybrid *Q. robur* x *Q. bicolor* Willd. is resistant to powdery mildew, while remaining receptive to the Burgundy truffle.

With these insights, we can proceed to consider the characteristics of a favorable Burgundy truffle orchard site, site preparation, the establishment and management of the truffière, and the eventual harvest and utilization of the truffles produced!

## Characteristics of a Good Burgundy Truffle Plantation Site

The suitability of a site for Burgundy truffle cultivation depends on several inter-related and equally important categories of factors: climate and position in the landscape; land-use history; soil properties; and access to water.

### Climate and Position in the Landscape

Analyses of climatic conditions conducive to Burgundy truffle fruiting have naturally focused on relationships between truffle harvests and annual patterns of air temperature and precipitation within the Burgundy truffle's native European geographic range (Table 3). Precipitation during the period June through September, as well as total annual precipitation, seems to be well correlated with annual Burgundy truffle harvests in France (Chevalier and



A *Quercus robur* seedling displaying a high level of powdery mildew on its foliage. Wire mesh is commonly used to protect young seedlings from rabbit damage.

Table 3. Mean precipitation and temperature for Cruzy, Burgundy, in France, and on the Swedish island of Gotland (Wedén et al., 2004b). Data for Missouri represent an orchard planting with established Burgundy truffle mycorrhizae.

Month	Monthly mean precipitation (mm)			Monthly mean temperature (C)			Main season for finding mature <i>Tuber aestivum</i> <sup>a</sup> fruiting bodies (exceptions exist)	
	Gotland	Burgundy	Missouri	Gotland	Burgundy	Missouri	Gotland	Burgundy
January	43.3	73.6	65.4	-1.1	2.7	-0.2		//////////
February	32.6	69.3	54.0	-1.8	3.6	0.9		
March	34.6	69.3	58.0	0.1	6.5	7.7		
April	29.3	60.4	75.1	4.0	9.0	14.5		
May	28.0	81.9	131.6	9.6	13.2	18.7		
June	39.5	75.9	136.7	14.3	16.0	23.2		
July	51.6	65.4	98.8	16.3	18.7	26.3		
August	48.5	66.4	108.6	15.9	18.6	25.8	/////	
September	56.8	72.0	60.9	12.1	15.1	20.0	//////////	/////
October	50.5	80.0	69.4	8.1	10.9	13.4	//////////	//////////
November	57.5	83.2	56.2	3.9	5.9	7.3	//////////	//////////
December	55.5	87.1	32.0	0.7	3.8	1.2		//////////

<sup>a</sup> French values are recalculated from m.e./100 g to %

Frochot 1997, Chevalier et al. 2001). Chevalier et al. (2001) estimate optimal monthly growing season precipitation in France to be: April, 20-50 mm (0.8-2 in); May, 60-80 mm (2.4-3.1 in); June, 60-80 mm (2.4-3.1 in); July, 50-100 mm (2-3.9 in); August, 60-80 mm (2.4-3.1 in); and September, 40-60 mm (1.6-2.4 in). In fact, greater precipitation during June through September seems to favor fruiting. As a case in point, heavy precipitation in July 2010 resulted in strong late-season fruiting in the Lorraine (personal communication, Dr. Christophe Robin and Mr. Jean-Sébastien Pousse). No comparable relationship to productivity is yet available for Gotland, where average May, June and August precipitation is lower than in Burgundy. Average precipitation April through September in central Missouri is greater than averages reported for Burgundy and Gotland.

The relationship between monthly air temperature and truffle harvest is not yet clear. Slightly lower average monthly temperatures on Gotland (Wedén et al. 2004b) result in earlier onset and conclusion of Burgundy truffle fruiting there than in France. In Missouri, April-October temperatures are warmer than in Europe, but the cooling effect of greater precipitation may mitigate the effect of higher air temperature. We can only speculate.

Manipulation of tree spacing and density permits the Burgundy truffle to be cultivated on modest slopes of almost any aspect (for example, Chevalier et al. 2001). For example, on gentle south-facing slopes, the shade provided by a greater tree density and/or closer spacing in east-west rows can mitigate the drying effects of warmer temperature and greater exposure to the sun.

The Burgundy truffle fungus does not tolerate poorly drained soils ("wet feet"), so flood plains are very inappropriate sites for trufficulture.

Slope positions below wooded areas may contain especially high inoculum levels ("spore banks") of competitor ectomycorrhizal fungi, since both overland and groundwater drainage of uphill wooded areas would certainly carry spores into the landscape below. The two other main factors contributing to spore banks of ectomycorrhizal fungi on potential truffle plantation sites are: 1) the extension of ectomycorrhizal tree roots for 50-meters or more into adjacent open areas; and 2) the fecal deposits of animals (for example, deer and burrowing

rodents) that feed on various ectomycorrhizal mushrooms (for example, Ashkannejhad and Horton 2006).

## Land-use History

It has been strongly and justifiably recommended that truffle orchards be established on land that has not been occupied by ectomycorrhizal tree species for many years. Soils associated with previous ectomycorrhizal forest vegetation are pre-infested with native ectomycorrhizal fungi well adapted to the site and likely to compete effectively with the truffle fungus. Because the roots of trees extend into open areas well beyond the extent of their canopies, it has also been recommended that truffle orchards be surrounded by a 50-meter buffer of previously unforested land.

Throughout the south-central U.S., a great deal of forested land was cleared during the early 20th century and converted at least temporarily to agriculture. Soil quality and site productivity varied dramatically. Poorer sites were either abandoned or grazed, and better sites were often fertilized and cropped. In contrast to conventional agriculture, truffle cultivation is most often successful on poor quality soils that have not been routinely fertilized. The intensity of root colonization by truffle fungi is inversely related to soil fertility; seedlings in fertile soils are capable of resisting infection by mycorrhizal fungi.

The presence of legumes may be a source of nitrogen-fixing bacteria for truffle development, but it remains to be determined whether the same strains of bacteria that fix atmospheric nitrogen efficiently in legume nodules are also effective in association with the Burgundy truffle fungus. The specificity of bacterial strains with different legumes is well known (Thrall et al. 2005), so truffle fungi also may perform best with their own uniquely adapted bacterial strains. Greenhouse trials with bacterial strains isolated from the Burgundy truffle fungus need to be conducted.

## Soil Properties

Much of what we know about appropriate soils for effective Burgundy truffle cultivation is based on studies conducted within the native (European) ranges of this truffle species. Yet we also know that some truffle species have been very successfully cultivated on rather different soils outside of Europe. Experience in New Zealand has demonstrated that

Table 4. Ranges of soil characteristics for productive Burgundy truffle sites in France (Chevalier and Frochot, 1997), and on the Swedish island of Gotland (Wedén et al., 2004b). Data for Missouri represent an orchard planting with established Burgundy truffle mycorrhizae.

Measured parameter	Sweden		France		Missouri
	Mean	Range	Mean	Range	Range
Clay <2 µm %	19.3	10.4-32.6	34.1	13.6-52.8	10-15
Silt 2-50 µm %	25.1	9.8-64.7	48.3	17.3-67.4	65-70
Sand 50-2000 µm %	55.6	12.9-79.8	17.5	2.8-69.1	17.5-22.5
Water pH	7.5	6.8-7.9	7.6	7.1-8.0	7.7-8
CaCO <sub>3</sub> (total) %	3.0	0.1-10.5	16.7	0.4-52.0	
Exchangeable calcium <sup>a</sup> (Ca) %	0.67	0.36-1.07	0.51	0.28-0.79	1.2-2.2
Assimilable phosphorus (P <sub>2</sub> O <sub>5</sub> ) %	0.020	0.002-0.120	0.009	0.002-0.082	0.001-0.002
Exchangeable magnesium <sup>a</sup> (Mg) %	0.019	0.009-0.045	0.017	0.005-0.041	0.008-0.011
Exchangeable potassium <sup>a</sup> (K) %	0.023	0.008-0.063	0.059	0.025-0.104	0.013-0.018
Ca/Mg	40.6	12.4-67.7	58.5	19.5-116.5	15-22.7
K/Mg	1.2	0.3-4.0	4.5	1.3-8.1	1.5-2.3
Organic matter %	11.9	6.0-21.2	9.7	4.4-21.1	2.5-3.6
Organic carbon %	6.9	3.5-12.3	5.6	2.6-12.3	1.5-2.1
Organic nitrogen %	0.54	0.3-1.1	0.46	0.3-0.8	0.2-0.3
C/N ratio	13.0	9.7-18.2	11.9	8.9-20.4	6.3-9.5

<sup>a</sup> French values are recalculated from m.e./100 g to %

the Périgord truffle is capable of very productive development on soils that are either clayier or sandier than most productive European soils (Wedén et al. 2004b, Hall et al. 2007). We have much to learn about the breadth of soil characteristics to which the Burgundy truffle is adaptable. Table 4 presents soil characteristics of productive Burgundy truffle sites in France (Chevalier and Frochot 1997) and on Gotland (Wedén et al. 2004b), in comparison with a typical Missouri River hills site (Bruhn, unpublished).

Fine root colonization levels by mycorrhizal fungi (including truffle fungi) are often inversely related to soil levels of nutrients which are more efficiently extracted by the mycorrhizal mycelium than by non-mycorrhizal fine roots. In essence, when required nutrients are in adequate supply, the plant resists colonization by the mycorrhizal fungus. Such nutrients include the relatively immobile elements such as phosphorus and iron, and scarce micronutrients. In other words, pre-emptive fertilization can inhibit mycorrhiza

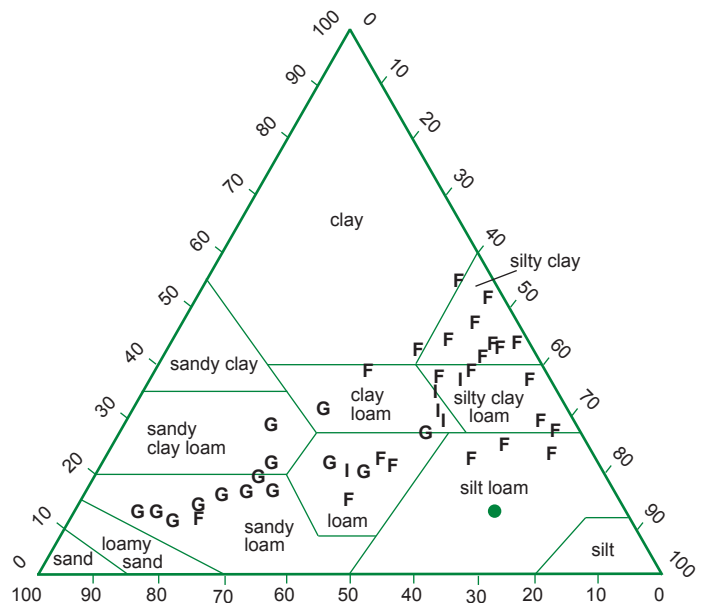


Figure 1. Soil texture triangle, indicating productive soils for the Burgundy truffle in Europe (F=France; G=Gotland; I=Italy) (Chevalier et al. 2001). The dark green dot represents UMCA's truffière soil (20% sand, 67% silt and 12% clay).



formation (and thus truffle production). This is why well-fertilized agricultural soils are not recommended as truffle plantation sites. Still, the Burgundy truffle fungus is deemed more tolerant than the Périgord black truffle of relatively high phosphorus and/or potassium levels (Chevalier et al. 2001).

In addition to the clear requirement for high pH associated with high soil levels of Ca and Mg, the Burgundy truffle thrives in soils with 9-12% organic matter, even as high as 20% (Chevalier et al. 2001, Wedén et al. 2004b). Fortunately, soil pH and organic matter level can be manipulated relatively easily and inexpensively with amendments of crushed limestone and various forms of organic matter. Appropriate ratios of Ca:Mg and K:Mg can be approximately obtained by judicious selection of agricultural crushed dolomitic lime sources from within the region.

Technically, a soil's pH value is its relative acidity, defined as the negative logarithm of the hydrogen ion concentration. A pH value of 7.0 is neutral (neither acidic nor alkaline). Soils with pH values below 7.0 are increasingly acidic (higher concentrations of hydrogen ions), whereas pH values greater than 7.0 indicate increasing alkalinity (lower hydrogen ion concentrations as a result of higher calcium, magnesium and potassium ion concentrations). Unlike most agronomic crops, the Burgundy truffle fungus clearly requires a soil pH above 7.0 for fruiting (Riousset et al. 2001).

Nutrient-holding capacity is directly related to a soil's clay and organic matter content. The Burgundy truffle thrives with moderate levels of clay and organic matter (Chevalier et al. 2001, Wedén et al. 2004b), as long as the soil is well-aerated and freely draining (Riousset et al. 2001). Soil drainage can become restricted at high levels of clay content, but clay particles tend to aggregate at the high pH levels favored by the Burgundy truffle, improving soil aeration and drainage.

Figure 1 highlights the range of soil textures associated with productive truffle plantations known from France, Italy and Gotland (Chevalier et al. 2001). Despite significant overlaps the ranges of soil texture associated with Burgundy truffle habitat differ substantially among these three regions. Will data from new regions extend the range of soil textures

known to support Burgundy truffle production? The red lines demonstrate how to pinpoint the soil texture of a typical Missouri silt loam soil.

## Access to Water – Irrigation

Finally, plantation sites with access to water for seasonal irrigation have a distinct advantage over sites where irrigation is not feasible. We have already mentioned the relationship between growing season precipitation and truffle harvest (see “Climate and Landscape Position,” above), and the south-central U.S. often experiences a dry mid-summer period. It is widely believed that the Burgundy truffle fungus forms truffle mushroom primordia during the spring and summer. Unlike most other mushrooms, Burgundy truffles apparently develop slowly over a period of months before maturing to their full size and fragrance. Thus, for maximum productivity, growers should be prepared to supplement natural precipitation with overhead irrigation every two weeks from late spring through early autumn to maintain optimum levels of precipitation. (See “Irrigation,” under “Orchard Site Preparation,” below.)

*Top: Spring-form rakes are widely used for incorporation of lime and other amendments in truffle site preparation and maintenance. The disadvantage to this tool is that it drags roots through the soil, which can spread unwanted competing mycorrhizal fungi around the plantation. Middle and bottom: Two implements useful in establishing and maintaining Burgundy truffle plantations in the Lorraine, France. Middle: The comb at the top of the blade is used to clear heavy grass or brush, and the blade is used to aerate the soil to 2-ft. depth in preparing the planting bed. A key feature of the tool is the two side wings that loosen the soil laterally in addition to the vertical slicing accomplished by the blade. One result is that no air pockets are left in the soil. Bottom: This device is used in the very early spring to regenerate fine roots in the plantation. The 8-in. teeth accomplish both aeration and disturbance of lateral roots. New fine roots grow into the disturbed upper soil as quickly as two months after treatment. In contrast to the spring-form rake, this tool is not dragged across the plantation; instead it is repeatedly moved and rocked to accomplish slicing without dragging.*



## Plantation Site Preparation

Once an appropriate orchard site has been selected, the first steps in truffle cultivation are to make any desirable soil modifications and to install other useful infrastructure.

### Soil Amendment

The most critical soil modification is the adjustment of soil pH by the addition of lime to the upper 30 cm (12 in) of soil. Raising the pH into the appropriate range may however affect the availability of other important plant nutrients (for example, iron, magnesium, phosphorus and trace elements). For this reason, there are nutritional advantages to using crushed dolomitic limestone, especially “reddish” forms that contain substantial quantities of iron as well as magnesium. Generally, amendment of levels of iron, phosphorus and trace nutrients (if necessary) may be best made after plantation establishment, based on analysis of foliar nutrient content, if nutrient deficiency is evident (Hall et al. 2007).

A rule of thumb suggests that approximately 1-1.5 tons per hectare of finely crushed limestone are required to raise the pH of a soil by 0.1 pH unit for each 10 cm of soil depth being treated (Hall et al., 2007). Additional chip-sized limestone (0.5-1 ton per hectare) can be added to provide buffering into the future. Because it may take up to a year for soil pH to equilibrate after liming, it may be necessary to apply lime more than once in preparing the plantation site, and because the lime needs to be thoroughly mixed into the soil, trees should not be planted until an acceptable pH level (at least 7.5) has been achieved. The most appropriate form of lime is generally a crushed dolomitic limestone, containing substantial levels of magnesium (and ideally iron) as well as calcium, because the calcium/magnesium balance is important to plant nutrition, and because iron becomes less available to plants as the pH increases. If sufficient iron is not naturally available (based on appearance of characteristic symptoms of stunted growth and yellowing foliage), it can be applied as a supplemental foliar spray.

Liming materials are available in several forms, which vary in their nutritional content and rate of incorporation into the soil. Agricultural or pelletized lime (pel lime) incorporates into the soil more rapidly than dolomitic lime but does not contribute significantly to levels of iron, magnesium or other minerals. Depending on various factors, it may take

a year for soil pH to equilibrate after incorporation of a dolomitic lime treatment into the upper 1-ft (30-cm) of soil. Because currently available models of pH response to lime treatments are unreliable at pH values above 7.0, it's best to modify orchard site soil pH in stages so as not to raise soil pH above 8.0. Thus, one should plan for orchard soil preparation to take at least two years. There are many different kinds of implements that can be used to incorporate soil amendments. The objective is to incorporate amendments as uniformly as possible without destroying soil structure. Too many passes over a site can “polish” the interface between the amended soil and the subsoil, resulting in poor drainage. Because each lime application needs to be incorporated fairly uniformly into the soil, amendment needs to be completed before infected trees are planted on the site to avoid damaging developing root systems. The spring-form rake depicted in Figure X is commonly used to incorporate supplemental lime into plantation soil.

### Weed Management

Existing vegetation on orchard sites competes with truffle seedling root systems for space, water and nutrients. On the other hand, short-lived plant roots contribute organic matter while loosening soil structure, and legumes contribute valuable nitrogen to the soil. Other plants may contribute to development of a Burgundy truffle ecosystem in ways still unknown.

Nevertheless, too much weedy vegetation can acidify the soil as organic matter decomposes to produce weak acids. Thus it is common practice to reduce competing vegetation prior to plantation establishment. Vegetation control can be accomplished using mulching materials, herbicides, grazing animals or by very close mowing. The herbicides Roundup® (glyphosate) and Buster® (glufosinate-ammonium) seem to be well tolerated by the Burgundy truffle fungus (Chevalier et al. 2001) though care must be taken not to let these herbicides drift onto tree foliage! The effects of many different mulching materials and fabrics are yet unclear but it has been demonstrated that the Burgundy truffle can benefit from vegetation control using black, water-permeable mulching fabric (Zambonelli et al. 2005). Mowing has the twin disadvantages of being time consuming while also contributing to soil compaction over time.

Weedy tree species on an orchard site need to be treated according to their mycorrhizal character (EM or AM). Because the tree species most commonly associated with the Burgundy truffle (hazels and oaks) are EM, the presence of AM trees does not pose a threat of mycorrhizal fungus competition to the truffle fungus, and the AM trees may contribute useful shade. If direct competition between AM and truffle trees is considered problematic, the AM trees can simply be removed from the site. On the other hand, EM trees in the vicinity of an orchard should be treated differently. When possible, orchards should be located at some distance from natural EM woodlands to reduce competition between native EM fungi and the cultivated truffle fungus. If native EM trees occur on an orchard site, it's preferable to cut them off and poison the stump rather than trying to remove the stump and root system from the soil. Invariably, efforts to remove root systems leave mycorrhizal fine roots in the soil and have the effect of spreading EM fungi within the orchard site. Table 4 presents a list of tree genera commonly associated with AM or EM fungi.

## Irrigation

Truffle orchard development and fruiting both benefit from adequate soil moisture year-round. While truffle fungi do not like “wet feet,” developing truffles are damaged during prolonged dry periods. The mid-summer period is particularly critical in the truffle life cycle because young truffles become independent of their originating root system and are especially vulnerable to desiccation.

### *Overhead irrigation is much superior to drip irrigation because:*

- 1) It provides moisture to truffles wherever they may be developing;
- 2) It favors the development of a wider-spreading root system.

Missouri climates commonly feature a dry summer period. It is advisable to provide the equivalent of a summer shower to prevent dry periods of more than two weeks' duration. (See “Climate and Landscape Position” under “Characteristics of a Good Burgundy Truffle Plantation Site,” above.) Both precipitation and irrigation have the additional benefit of moderating high summer soil temperatures.

## Fencing

Perhaps the final element of orchard site preparation is the fencing of the area for exclusion of unwanted animals (both two- and four-legged species). Fences are generally warranted where trespass by deer, livestock, feral hogs or people are a concern. Fences for exclusion of deer are commonly 10 ft. tall, constructed of heavy wire with a closer mesh at the bottom for exclusion of smaller animals (goats, sheep, rabbits). Feral hogs can be discouraged from digging under the fence by extending it approximately 2 ft. below ground, curving outward from the orchard up to 3 ft. There may be some added advantage to installing strands of solar-powered electrified wire 1-3 ft. above ground, 1 ft. outside the fence. The illustration below presents a fence of this type.



*Deer- and hog-proof fence surrounding the Burgundy truffle truffière*

*site at the Maison de la Truffe museum in Boncourt-sur-Meuse, the Lorraine, France. A unique feature is that the fence extends 0.5 meters below the soil surface, curving outward from the truffière for about 1 meter. The below-ground extension of the fence prevents all but the most determined animals (badgers, for example, see inset photo) from burrowing into the truffière.*

## Plantation Establishment

One major difference between the preferred habitats of the Burgundy vs. Périgord truffles involves openness of the orchard floor. Périgord truffle development is favored by full access of sunlight to the orchard floor, whereas fruiting by the Burgundy truffle is favored by substantial shade and development of a litter layer. In fact, it is widely

believed that the Burgundy truffle fruits poorly if at all until canopy closure occurs. As a result, there is a longer interval between planting and harvest of Burgundy truffles than is the case with Périgord truffles (approximately eight vs. five years, respectively).

## Plant Density

The planting design and tree density in a truffle plantation needs to take into consideration the species of truffle being cultivated, the tree species selected, and site characteristics. Further, plantation tree density and tree species composition may need to be adjusted as the plantation matures.

Because the Burgundy truffle benefits from shade and a litter layer, tree densities will generally be much higher in Burgundy truffle plantations (perhaps 400-1200 trees/ha) than in plantations of the Périgord truffle which thrives with full access of sun to the soil (400 trees/ha or fewer) (Chevalier et al. 2001). Tree density can be reduced as trees grow and begin to cast shade. Perhaps the most obvious approach would be to plant infected seedlings more densely, but infected seedlings are quite expensive and would eventually need to be thinned. An alternative approach would be to interplant truffle inoculated trees among previously-planted arbuscular mycorrhizal “shelter trees” (see Table 1), whose sole purpose is to provide early shade to hasten the onset of fruiting (Wehrle et al. 2009).



*A typical Burgundy truffle plantation (mostly European hazel, inset), located in Boncourt-sur-Meuse (the Lorraine, France).*



Because hazels are generally smaller and shorter-lived than oaks, yet begin to produce truffles at an earlier age than oaks, it is possible to alternate oaks and hazels within plantation rows with the intention of gradually removing hazels as the plantation matures (Hall et al. 2007).

Tree densities in Burgundy truffle plantations should be lower under conditions favoring rapid tree growth and/or larger tree stature (Chevalier et al. 2001), such as fertile and/or deep soils, access to irrigation, and in plantations of tree species which attain greater stature at maturity (oaks compared to hazels, for example). Plantation density can also be lower on north-facing slopes than on other aspects (due to greater natural shade).

## Companion Plantings

Weeds have been loosely defined as plants that are growing where you don't want them. However, a number of woody and herbaceous plant species are suspected of contributing to truffle habitat and nutrition.

It has recently been shown that a variety of bacteria related to those that fix nitrogen in legume nodules occur in large numbers within truffle fruit bodies. In our ongoing studies of bacterial communities within Burgundy truffle fruit bodies, we have recently isolated into pure culture nitrogen-fixing bacteria closely related to strains of *Bradyrhizobium* and *Sinorhizobium meliloti* commonly used in agriculture. Italian colleagues have now demonstrated nitrogen fixation within fruit bodies of *T. borchii* Vitt. (another truffle species) by the acetylene reduction technique (Barbieri et al. 2010). This raises the question of whether or not the nitrogen produced by these bacteria is essential to mycorrhizae formation and/or fruiting of truffle fungi. If the



*A very productive, centuries-old hazel grove on Gotland. Note the closed canopy that seems to be a requirement for abundant Burgundy truffle fruiting.*

answer is “yes,” then modest populations of nitrogen-fixing hosts might ensure the well-distributed presence of these bacteria across a truffle orchard site. It may also prove advantageous to simultaneously inoculate seedlings in the greenhouse with selected bacterial strains as well as the desired truffle fungus.

## Planting

There are many ways to “put a tree in the ground”! But in establishing a truffle plantation, we are planting precious tree seedlings painstakingly produced to be well-colonized by the Burgundy truffle fungus into ground that has been carefully prepared to support truffle development. Wouldn't it be a shame to plant these expensive trees in any sub-optimum manner? So what do we know about properly planting truffle seedlings?

We can learn from a technique that is currently finding favor in France. In this technique, developed jointly by the French INRA and equipment manufacturer Claude Becker (Toul, France), planting beds 1 meter wide are prepared along the slope contour using Becker's “culti-sous-soleur” tool (see center image, pg. 9). The comb above the blade is first used to rake away larger herbaceous vegetation and brush. The winged blade is then used to loosen the soil to a depth of 0.6 meter without overturning the soil horizons. Three strokes are made with the blade; the first stroke defines the center of the row. The second and third strokes are made parallel to the first, 0.5 meters to each side with the blade angled to bring soil toward the center, forming a raised bed 0.2-0.4 meters high. An important feature of this method is that strokes are discontinuous along the planting bed, resulting in minimal dragging of debris (roots, etc.) across the site. A skilled operator can produce 100 meters of planting bed, 1 meter wide, per hour.

While this tool will not be available everywhere, any planting bed preparation technique that emulates this effect would be helpful.

Once the raised planting bed has been formed, it is important to plant seedlings at the appropriate depth. Oaks and hazels should be planted with the soil level approximately at the point of attachment of the acorn or nut.

Rabbits and mice can cause serious damage to young seedlings by eating twigs and/or girdling seedlings (eating the tender bark). In areas where rodents

abound, seedlings need to be protected by mesh or solid tubes (for example, Wedén et al. 2009).

## Plantation Maintenance

Due to the broader habitat tolerance of the Burgundy truffle, plantation maintenance is somewhat less involved for the Burgundy truffle than for the Périgord truffle, but the following concerns are very real.

### Managing Compaction

Because the Burgundy truffle prefers well-aerated soils, it is important to minimize soil compaction in the process of establishing and maintaining the truffle orchard. This can be accomplished: 1) by using lightweight equipment in plantation management; 2) by using equipment with balloon tires for broader weight distribution; and 3) by minimizing the number of equipment passes over time through the plantation.

### Soil Cultivation and Supplemental Liming

Soil cultivation is used to incorporate lime or other supplemental fertilizer, and to loosen soil structure to facilitate truffle growth and size. However, cultivation should only be practiced sparingly to avoid compaction, and in a manner that avoids dragging roots contaminated by competing ectomycorrhizal fungi throughout the plantation (Chevalier et al. 2001). When necessary, cultivation should be accomplished in the early spring, as soon as the soil is dry enough to minimize compaction, yet prior to the initiation of new mycorrhizas (in other words, as early as late February or March, if possible).

The “pioche herse,” developed by Claude Becker of Toul, France (see bottom photo, page 9), cultivates the soil to a depth of 20 cm (8 in) by repeatedly picking up, setting down, and rocking the plate with six teeth in the soil while avoiding any dragging affect. Others have used roto-tiller-like equipment with blades bent vertical to sever roots to 10-cm depth in order to refresh truffle mycorrhizas, loosen the soil and improve productivity.

One persistent concern that may be addressed by soil cultivation (Chevalier and Frochot 1997) is the deleterious effect of Burgundy truffle initiation too close to the soil surface. Truffles that form too close to the soil surface may be more easily damaged by insects and/or desiccation. Loosening the soil

to 10 or 20 cm may encourage deeper rooting and consequently deeper fruiting by the truffle fungus.

On plantation sites where it was necessary to add lime to raise the soil pH during initial site preparation, several factors result in gradual soil acidification. These factors include the gradual leaching of calcium and magnesium, and the decomposition of organic matter to form organic acids. Without the buffering effect of naturally occurring limestone, it will eventually become necessary to add additional agricultural lime (perhaps once per decade) to maintain the soil pH at 7.5 or greater. This can be accomplished in conjunction with early spring soil cultivation.

## Water Management

It is useful to think of the life of a truffle plantation as comprising two phases: an establishment (pre-fruiting) period; and a mature, productive period. Irrigation during the pre-fruiting period is designed to support development of a well-colonized root system throughout the orchard, whereas irrigation during the productive period is more concerned with the survival and growth of the truffle fruit bodies.

Because truffle fruit bodies develop over a period of months, and developing truffles are quite susceptible to desiccation, adequate soil moisture needs to be maintained throughout the growing season (Chevalier et al. 2001, Rioussset et al., 2001). In the south-central U.S., it will be important to supplement natural precipitation during periods of summer drought. A useful rule of thumb is to irrigate every two weeks in the absence of natural rainfall, targeting optimum monthly summer precipitation values. (See "Climate and Position in the Landscape," pg. 6). Keep in mind, however, that excessive irrigation is worse than no irrigation at all (Chevalier et al. 2001).

## Weed Management

Weed management can be a more or less persistent concern during the orchard establishment period, but as canopy closure occurs in Burgundy truffle orchards, weed populations usually decline. As

the Burgundy truffle spreads with the developing root systems, its mycorrhizae have a somewhat allelopathic effect on surrounding vegetation, producing a characteristic "burnt" zone (with reduced vegetation) on the plantation floor.

During plantation establishment (pre-fruiting), a variety of mulching materials can be used to suppress weed growth. Zambonelli et al. (2005) found that black water-permeable weed barrier fabric served well and favored Burgundy truffle establishment. Alternatively, the herbicides Roundup® (glyphosate) and Buster® (glufosinate-ammonium) seem to be well-tolerated by the Burgundy truffle fungus, though care must be taken not to let these herbicides drift onto tree foliage!

## Pruning

Strategies differ substantially for pruning/shaping the trees in Burgundy vs. Périgord truffle plantations. Pruning strategies for the Burgundy truffle focus on early production of shade, the exact opposite of the focus in Périgord truffle plantations. Yet even though shade is the early objective, so also is access to the plantation floor for cultivation and eventual harvest activity.

## Harvesting

Interest in truffles dates back to antiquity. Greek and Roman philosophers discussed the nature and origins of truffle fruiting bodies. It was variously suggested



*Humans have long depended on animals to detect the location of truffles. People have relied on the natural relation-*

*ships of truffles with pigs and even with certain winter-active flies. More recently, dogs have become most popular because they are easily trained and less interested in eating the truffles. The breed of dog does not matter as much as the individual dog's ability to focus on the task at hand and its eagerness to please its master.*



Above: This Burgundy truffle (inside the circle) formed just below the soil surface at the base of a cluster of European hazel stems on the Baltic island of Gotland (Sweden). Bottom inset: A collection of Burgundy truffles fresh from the woods before cleaning with a toothbrush. Top inset: This truffle (inside the circle) formed 6 inches below the soil surface. The tool used to excavate it is one of many designs; but more important than the design is the care taken in using it so as not to damage the truffle during excavation.

that truffles originated during thunderstorms when lightning struck damp earth or were somehow the work of the devil. It's hard to know exactly how or when human attention was first drawn to truffles, but it almost certainly involved observations of wild animals searching naturally for these delicacies.

Perhaps human attention was first drawn to the Burgundy truffle through observation of the rooting activities of wild hogs, which are naturally attracted by the truffle fragrance. It is well known that one of the principal volatile compounds produced by ripe truffles is very similar to one of the mating hormones produced by male hogs. As a result, the first domesticated animals used to search for truffles were undoubtedly female hogs. Unfortunately, hogs appreciate the flavor of truffles at least as much as do humans, so it was very important to muzzle your pig before you arrived at the truffle site. Also, it could be difficult to keep the whereabouts of your special truffle patch secret if you were seen transporting a pig.

A more difficult method of searching for truffles depends on observing the presence of several species of fungus fly that remain active during cold weather and lay their eggs in the vicinity of ripe truffles. This method can work well when searching an area where truffles are known to occur. With this method, the truffle hunter walks very slowly, facing the sunlight (to avoid casting a forward shadow), perhaps gently waving a stick, hoping to spot the presence of any truffle flies. Once a truffle fly has been flushed from the soil surface, the hunter can search for tell-tale cracks in the soil resulting from the rapid growth of a truffle. The truffle hunter may also resort to sniffing a handful of soil to verify the presence of a truffle before carefully searching with a trowel-like tool.

By the 17th century, for several reasons, dogs replaced hogs as the truffle-hunting animal of choice. Most dogs have a sense of smell adequate to detect the presence of a Burgundy truffle 6 inches below ground at a considerable distance. The main factors seem to be the dog's attention span and eagerness to please its master. Experienced truffle hunters have their individual preferences of breed: Labrador retrievers, Brittany spaniels, poodles, mixed breeds, etc. There is even a highly respected breed, the Italian lagotto romagnolo, which has been developed specifically for truffle hunting.

There are various methods for training dogs to search for truffles. Some people train their dogs by feeding them truffles, but this has the undesirable result of reducing the harvest. It's far better to train a dog with a unique but different reward that is only given for the discovery of a truffle in the field. Typically dogs will be trained using a very limited and unique vocabulary that the dog associates solely with the truffle hunt. Words such as "Cherche!" or "Syk!" (Search!), "Ou est la truffe?" (Where's the truffle?), etc. During the off-season, dogs can be trained to find objects flavored with commercially available truffle oil.

The commercial value of a truffle is closely linked to its fragrance and maturity. All the animal-based methods presented above depend on truffle fragrance and thus have the advantage of discovering only truffles that have at least begun to mature. Once a dog has indicated the location of a truffle, the animal's attention needs to be diverted so as not to damage the truffle. The hunter then uses a metal tool to gently probe the soil in search of the buried

treasure. If the truffle is not found close to the soil surface, the hunter may hold a handful of soil to the nose in an effort to confirm the tell-tale fragrance of a nearby truffle. The hunter also may ask the animal to reaffirm the truffle's location. The fragrance of a ripe truffle can travel through the soil by means of worm tunnels or cracks, causing the dog to mis-identify the location of the nearby truffle.

In cultivating valuable truffle species, one should be prepared to discover fruiting by other truffle species native to the area (Chevalier and Frochot 1997; Pruett et al. 2008; Bruhn et al. 2009; Wedén et al. 2009). Field guides exist that help identify these native truffle species (Trappe et al. 2007, Trappe et al. 2009). One shouldn't feel discouraged to learn that one's plantation is shared by native truffle species. It would be unrealistic to expect the Burgundy truffle to be the only truffle species well adapted to one's plantation site. The important point is to keep track of the survival of the Burgundy truffle fungus in the plantation. However, studies with the Italian white truffle (*T. magnatum* Vitt.) have shown surprisingly that successful fruiting does not depend on *T. magnatum* dominance of the mycorrhizal community (Bertini et al. 2006).

In some places truffles are harvested by such means as raking the forest floor (without the selective benefit of an animal's nose). This results in the harvest of truffles in all states of maturity (and value), and may even damage the forest floor.

### ***Cooking with truffles: Advice on fundamentals***

- It is preferable to eat truffles fresh.
- Truffles are a condiment; not just a decoration.
- Truffles lose their fragrance with prolonged or excessive heat. Different species of truffles have different degrees of thermolability.
- To impart the best fragrance to a dish, capture the truffle's fragrance in a fatty substance: butter, cream, cheese, egg yolks, oil.
- Capture of fragrance requires at least overnight.
- Fats of animal origin best capture truffle fragrance.
- Use simple preparations that avoid other strongly flavored ingredients.
- Plan for 1/3-1/2 oz. Burgundy truffle per serving.

## **Recipes**

### ***Burgundy Truffle Omelette*** ***Christina Wedén***

6 medium-size Burgundy truffles  
1 dozen large fresh eggs  
1 cup whole milk  
Olive oil for cooking  
Salt and pepper to taste

1 day in advance: Place 5 truffles and the fresh eggs in a large glass jar in the refrigerator.

Grate 1 truffle into the milk in a glass jar, and refrigerate.

At the last minute: Beat eggs with truffle-infused milk, and pour into pan lubricated with olive oil over low heat, and cover.

Just before folding, place thin slices of the 5 truffles down the center of the omelette.  
Serve immediately.

### ***Burgundy Truffle Butter*** ***Anonymous***

One-quarter pound butter  
As much as one-half the volume in fresh Burgundy truffle

1 day in advance: Grate the truffle into the butter at room temperature.

Mix thoroughly and refrigerate.

At the last minute: Toast slices of white baguette.

Spread with truffled butter.

Top with a thin slice of fresh truffle if available, and serve immediately.

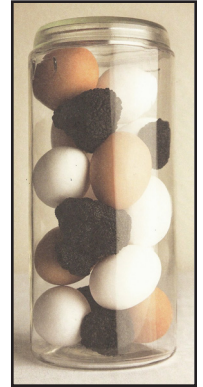
### ***Burgundy Truffled Brie*** ***Anonymous***

A wedge of brie  
Sliced truffle  
Water crackers or sliced white baguette

1 to 7 days in advance: Slice brie in half horizontally.

Cover lower half of brie with a single complete layer of thinly-sliced truffle.

Replace upper half of brie, enclose in plastic or glass, and refrigerate.





At the last minute: Allow truffled brie to come to room temperature.

Slice onto warm crackers or toasts, and serve immediately.

### **Fresh-frozen Truffled Olive Oil Toasts** **Christine Fischer**

Fresh truffles dipped in olive oil and frozen  
Olive oil for infusion  
White bread toasts

1 day in advance: Thaw truffles, slice into olive oil, and refrigerate.

At the last minute: Bring olive oil and truffle slices to room temperature.

Toast white bread, brush with truffled olive oil and top with a thin truffle slice.

Serve immediately.

## **Additional Resources on Truffle Biology, Cultivation, and Cuisine**

### **Cookbooks**

Czarnecki, J. 1995. *A Cook's Book of Mushrooms*. Artisan. New York.

Hurst, J., and L. Rutherford. 1991. *A Gourmets Guide to Mushrooms and Truffles*. HP Books, Los Angeles.

Meunier, G., and Y. Schweitzer. 2010. *Les Truffes de Lorraine*. Lorraine Consell Régional. (in French).

Wheeler, S. 1999. *The Complete Mushroom Cookbook*. Anness Pub., Ltd, London.

### **Internet Resources: Consultants and Information**

Agri-Truffe. <http://www.agritruffe.eu/>

Garland Truffles. <http://www.garlandtruffles.com/>

New World Truffières, Inc. <http://truffletree.com/>

### **Internet Resources: Events**

The Oregon Truffle Festival. <http://www.oregontrufflefestival.com/>

### **Internet Resources: Forest Management**

University of Missouri Forestry Extension: <http://extension.missouri.edu/explore/agguides/forestry>

Forest Management for Landowners, Missouri Department of Conservation: [www.mdc.mo.gov/forest/library/](http://www.mdc.mo.gov/forest/library/)

Optimum site conditions for North American tree species, United States Forest Service: [www.na.fs.fed.us/spfo/pubs/silvics\\_manual/table\\_of\\_contents.htm](http://www.na.fs.fed.us/spfo/pubs/silvics_manual/table_of_contents.htm)

### **Literature**

Allen, S.C., S. Jose, P.K.R. Nair, B.J. Brecke, P. Nkedi-Kizza, and C.L. Ramsey. 2004. Safety-net role of tree roots: Evidence from a pecan (*Carya illinoensis* K. Koch)-cotton (*Gossypium hirsutum* L.) alley cropping system in the southern United States. *Forest Ecology and Management* 192:395-407.

Ashkannejhad, S., and T.R. Horton. 2006. Ectomycorrhizal ecology under primary succession on coastal sand dunes: Interactions involving *Pinus contorta*, soil-loid fungi and deer. *New Phytologist* 169:345-354.

Barbieri, E., L. Bertini, I. Rossi, P. Ceccaroli, R. Saltarelli, C. Guidi, A. Zambonelli, and V. Stocchi. 2005. New evidence for bacterial diversity in the ascoma of the ectomycorrhizal fungus *Tuber borchii* Vittad. *FEMS Microbiology Letters* 247:23-35.

Barbieri, E., C. Guidi, J. Bertaux, P. Frey-Klett, J. Garbaye, P. Ceccaroli, R. Saltarelli, A. Zambonelli, and V. Stocchi. 2007. Occurrence and diversity of bacterial communities in *Tuber magnatum* during truffle maturation. *Environmental Microbiology* 9:2234-2246.

Barbieri, E., P. Ceccaroli, R. Saltarelli, C. Guidi, L. Potenza, M. Basaglia, F. Fontana, E. Baldan, S. Casella, O. Ryahi, A. Zambonelli, and V. Stocchi. 2010. New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*. *Fungal Biology*, doi:10.1016/j.funbio.2010.09.001.

Bencivenga, M., D. Donnini, M. Tanfulli and M. Guiducci. 1995. Tecnica di campionamento delle radici e degli radicali per valutazione delle piante micorrizzate. *Mic. Ital.*, 2: 35-47.

Bertini, L., I. Rossi, A. Zambonelli, A. Amicucci, A. Sacchi, M. Cecchini, G. Gregori, and V. Stocchi. 2006. Molecular identification of *Tuber magnatum* ectomycorrhizae in the field. *Microbiological Research* 161:59-64.

Bonfante, P., and I.-A. Anca. 2009. Plants, mycorrhizal fungi, and bacteria: A network of interactions. *Annual Review of Microbiology* 63:363-383.

Bruhn, J.N., G.E. Pruett, and J.D. Mihail. 2009. Progress toward truffle cultivation in the central USA. 5th International Workshop on Edible Mycorrhizal Mushrooms, Chuxiong, Yunnan, PRC, 27-29 August 2007. *Acta Botanica Yunnanica, Supplement XVI* :52-54.

- Cerigini, E., F. Palma, E. Barbieri, M. Buffalini, and V. Stocchi. 2008. The Tuber borchii fruiting body-specific protein TBF-1, a novel lectin which interacts with associated Rhizobium species. *FEMS Microbiology Letters* 284: 197-203.
- Chevalier, G., and J. Grente. 1978. Application pratique de la symbiose ectomycorrhizienne: production à grande échelle de plants mycorrhizés par la truffe (*Tuber melanosporum* Vitt.). *Mushroom Science*, 10: 483-505.
- Chevalier, G., and H. Frochet. 1997. La Truffe de Bourgogne. Editions Pétrarque, Levallois-Perret (in French).
- Chevalier, G., Gregori, G., Frochet, H., and A. Zambonelli. 2001. The cultivation of the Burgundy truffle. In: Hall, I. (Eds.). *Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms*. 12 p on CD. NZ.
- Delmas, J. 1978. Tuber spp. In: Chang, S.T., and W.A. Hayes (Eds.), *The Biology and Cultivation of Edible Mushrooms*: 645-681. New York: Academic Press, Inc.
- Fischer, C., and C. Colinas. 1996. Methodology for certification of *Quercus ilex* seedlings inoculated with *Tuber melanosporum* for commercial application. 1st International Congress on Mycorrhizae. Berkeley, CA. <http://labpatfor.udl.es/docs/tubeng.html>
- Frey-Klett, P., J. Garbaye, and M. Tarkka. 2007. The mycorrhiza helper bacteria revisited. *New Phytologist* 176:22-36.
- Hall, I.R., G.T. Brown, and A. Zambonelli. 2007. *Taming the Truffle: The History, Lore, and Science of the Ultimate Mushroom*. Timber Press, Inc., Portland, OR.
- Iotti, M., and A. Zambonelli. 2006. A quick and precise technique for identifying ectomycorrhizas by PCR. *Mycological Research* 110:60-65.
- Müller, W.R., T. Rauscher, and R. Agerer. 1996. *Tuber uncinatum* Chat. + *Corylus avellana* L. In Eds: Agerer, R., R.M. Danielson, S. Egli, K. Ingleby, D. Luoma, and R. Treu. *Descriptions of ectomycorrhizae* 1:179-183.
- Olivier, J.-M., J.-C. Savignac, and P. Sourzat. 2002. *Truffe et Trufficulture*. Editions Fanlac, Périgueux. (in French).
- Pruett, G.E., J.N. Bruhn, and J.D. Mihail. 2008. Temporal dynamics of ectomycorrhizal community composition on root systems of oak seedlings infected with Burgundy truffle. *Mycological Research* 112:1344-1354.
- Pruett, G.E., J.N. Bruhn, and J.D. Mihail. 2008. Colonization of pedunculate oak by the Burgundy truffle fungus is greater with natural than with pelletized lime. *Agroforest. Syst.*72: 41-50.
- Pruett, G.E., J.N. Bruhn, and J.D. Mihail. 2009. Greenhouse production of Burgundy truffle mycorrhizae on oak roots. *New Forests* 37:43-52.
- RiOUSset, L. et G., G. Chevalier, and M.C. Bardet. 2001. *Truffes d'Europe et de Chine*. INRA Editions, Paris (in French).
- Rocchia, J.-M. 1995. *Truffles, the black diamond and other kinds*. Editions A. Barthélemy, Avignon. (translation from French).
- Smith, S.E., and J.J. Read. 1997. *Mycorrhizal Symbiosis*. Academic Press, London.
- Thrall, P.H., D.A. Millson, A.C. Jeavons, M. Waayers, G.R. Harvey, D.J. Bagnall, and J. Brockwell. 2005. Seed inoculation with effective root-nodule bacteria enhances revegetation success. *Journal of Applied Ecology* 42:74-751.
- Trappe, M., F. Evans, and J. Trappe. 2007. *Field Guide to North American Truffles*. Ten Speed Press, Berkeley.
- Trappe, J.M., R. Molina, D.L. Luoma, E. Cazares, D. Pilz, J.E. Smith, M.A. Castellano, S.L. Miller, and M.J. Trappe. 2009. *Diversity, Ecology, and Conservation of Truffle Fungi in Forests of the Pacific Northwest*. USDA Forest Service Gen. Tech. Rep. PNW-GTR-772. <http://www.fs.fed.us/pnw/>
- Wedén, C., E. Danell, F.J. Camacho, and A. Backlund. 2004. The population of the hypogeous fungus *Tuber aestivum* syn. *T. uncinatum* on the island of Gotland. *Mycorrhiza* 14:19-23.
- Wedén, C., G. Chevalier, and E. Danell. 2004. *Tuber aestivum* (syn. *T. uncinatum*) biotopes and their history on Gotland, Sweden. *Mycological Research* 108:304-310.
- Wedén, C. 2008. *Tryffel*. Infotain & Infobooks Sweden AB, Stockholm (in Swedish).
- Wedén, C., L. Pettersson, and E. Danell. 2009. Truffle cultivation in Sweden: Results from *Quercus robur* and *Corylus avellana* field trials on the island of Gotland. *Scandinavian Journal of Forest Research* 24:37-53.
- Wehrle, L. 2008. *La sylvi-trufficulture et la truffe de Bourgogne: un nouveau pari qui concerne les forestiers*. INRA, ONF, RDV techniques n°r 22:68-72. (in French)

Wehrlen, L. 2009. Mieux planter! La technique "3B" élimine la végétation et décompacte le sol en une seule opération. INRA, ONF, RDV techniques n°r 25/26:7-12. (in French)

Wehrlen, L., G. Chevalier, G. Besançon, and H. Frochot. 2009. Truffle cultivation-forestry: A new strategy to produce the Burgundy truffle (*Tuber uncinatum* Chatin). 5th International Workshop on Edible Mycorrhizal Mushrooms, Chuxiong, Yunnan, PRC, 27-29 August 2007. *Acta Botanica Yunnanica*, Supplement XVI :97-99.

Zambonelli, A., M. Iotti, F. Zinoni, E. Dallavalle, and I.R. Hall. 2005. Effect of mulching on *Tuber uncinatum* ectomycorrhizas in an experimental truffière. *New Zealand Journal of Crop and Horticultural Science* 33:65-73.

### **DVDs**

Agroforestry Five-Practices DVD (Forest Farming section). The Center for Agroforestry at the University of Missouri. Available for purchase online at <http://www.centerforagroforestry.org/pubs/index.asp#dvd>

### **Organizations**

The North American Mycological Association: Includes an annual directory, a bimonthly newsletter, and the annual journal *Mycologia*. <http://www.namyco.org/>

The Mushroom Council: Marketing and consumer trend information. <http://www.mushroomcouncil.org/>

The North American Truffling Society. <http://NA-TRUFFLING.ORG/>



**Produced by The Center for Agroforestry  
at the University of Missouri**

Shibu Jose, Ph.D., Director  
203 ABNR Columbia, MO 65211



**Technology Transfer and Outreach Unit**

Michael Gold, Ph.D., Associate Director  
Larry D. Godsey, Ph.D., Economist  
Dusty Walter, Technical Training Specialist  
Julie Rhoads, Events Coordinator  
Michelle Hall, Sr. Information Specialist

For more information, visit [www.centerforagroforestry.org](http://www.centerforagroforestry.org)  
(573) 884-2874; [musnragroforestry@missouri.edu](mailto:musnragroforestry@missouri.edu)



**The Center for Agroforestry**  
University of Missouri

*A Global Center for Agroforestry, Entrepreneurship and the Environment*

This work was funded through the University of Missouri Center for Agroforestry under cooperative agreements 58-6227-5-029, 58-6227-2-008 and 58-6227-5-028 with the United States Dept. of Agriculture (USDA) Agricultural Research Service. Special recognition is given to the USDA, ARS, Dale Bumpers Small Farm Research Center, Booneville, Ark. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the USDA.