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Distinguishing the Périgord black truffle from the winter truffle and other look-alikes in the laboratory and field

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1 **Introduction**

If countries in the Southern Hemisphere are to become serious exporters of Périgord black truffle for Northern Hemisphere, out-of-season markets they will need to be able to produce a consistent supply of good quality truffles. This will require a well established method of ensuring the quality of the truffle and the development of large areas of productive truffières preferably free of other species of truffle that might be confused with the Périgord black truffle.

This report presents information on the characteristics and identification of Périgord black truffles and species that can be confused with them. Additional information can be found in the book “Taming the Truffle” by Ian Hall, Gordon Brown and Alessandra Zambonelli (2007).

2 **The Périgord black truffle and some look-alikes**

A range of truffles can be found on display in traditional European truffle markets. In the past this has included not only those truffles that can be legally sold there (see page 250 in Taming the Truffle) but also other species including some of Asiatic origin. The situation was further confused because more than one species of truffle were often sold mixed together (Figures 1 and 2).

*Figures 1 and 2. Lalbenque truffle market, December 2005. In the marketplace all that stands in the way of a good and a bad buy is the experience and honesty of the seller, the knowledge of the buyer and perhaps a Carte d’Identification Produit that lists the contents of a basket of truffles. The card below states that a basket of truffles contains a mixture of Périgord black truffles (T. melanosporum) and winter truffles (T. brumale).*
When truffles are sold covered in soil (which also adds to the weight!) one truffle can easily be mistaken for another (Figure 3). The soil might also cover up a multitude of other sins such as a little lead shot pushed into a truffle to increase weight and tooth picks used to join two truffles to make one bigger one. However, washing off the soil that coats the truffle decreases shelf life and so growers are naturally reluctant to do this until a sale has been made.

Figures 3 and 4. Périgord black truffles (Tuber melanosporum). In some parts of Europe truffles are still offered for sale covered with soil because it extends the shelf life. After washing it becomes easier to see the surface of the truffles which makes identification somewhat easier.
2.1  Distinguishing the Périgord black truffle from other species

There are a number of species that might be confused with the Périgord black truffle although with a little care, some knowledge, and a microscope mistakes can be avoided.

Figure 5. Four species of black truffles that might be confused by a novice. Clockwise from top left: Périgord black truffle (Tuber melanosporum), Chinese truffle (Tuber indicum), winter truffle (Tuber brumale) and Burgundy truffle (Tuber aestivum). Compare the broad white lines in the T. brumale truffles with the thin white lines in the T. melanosporum and Tuber indicum.

2.1.1  Périgord black truffle (Tuber melanosporum), Figures 3 - 9

The outer surface (peridium) of the Périgord black truffle is reddish brown when young turning brown then black when mature. The surface is ornamented with polygonal, 4-6 sided, slightly raised irregular warts, 2-5 mm across with the apex depressed and with radial groves (Figure 4). The contents of the truffle (gleba) are firmly attached to the underlying tissue. The contents are whitish when the truffles are young (Figures 6-8) then turning purple-black and finally jet black at maturity. The thin whitish veins that cross the contents of the truffle can become pinkish when exposed to the air (Figure 5). There are from 1-6 dark brown elliptical spores (20-) 25-55 μm x (15-) 20-35 μm (excluding ornamentation) in each spore sac (ascus, plural asci) which are densely ornamented by short spines 2.5-3 μm high often slightly curved towards the tips (Figure 9).
Figures 6-8. Young, immature Périgord black truffles have whitish contents. From top left: truffles harvested in early March 1997 in Alan and Lynley Hall’s truffle, Gisborne, New Zealand; immature, rejected truffles in Pébeyre’s establishment in Cahor, France, some showing where a small slice has been removed to reveal the contents of the truffle; and immature frozen truffles imported into New Zealand during the Northern Hemisphere off season.

Figure 9. Spores of the Périgord black truffle are covered with short spines which are usually curved at their tips. There can be 1 to 6 spores in each spore sac (ascus).
2.1.2 Winter truffle (Tuber brumale), Figures 10 and 11

The skin of the winter truffle is only loosely attached to the inside tissues and can be peeled away with a fingernail or by brushing (Figure 10). The warts on the surface are typically flattened and depressed at the apex. The contents of the truffle are a greyish brown, never with a purplish tinge and with sparse, broad, whitish veins. The elliptical spores are yellowish-brown at maturity, smaller than those of the Périgord black truffle (15-) 20-42 μm x 15-30 μm, and densely ornamented with straight, well spaced, pointed spines, 3-6 μm long (Figure 11).

Figures 10 and 11. Winter truffles (Tuber brumale) showing the fragile skin that is easily damaged (yellow arrows), the wide veins criss-crossing a greyish brown interior, and a spore ornamented with the characteristic long, straight spines.

2.1.3 Burgundy truffle (Tuber aestivum), Figures 12 - 14

Burgundy truffles are brown to black and 2 cm to more than 10 cm in diameter. The skin adheres to the tissues beneath and is ornamented with 5-7 sided pyramidal warts 3-9 mm wide, with longitudinal fissures and some fine transverse markings. When mature the inside of the truffle is a dark brown. The thin white veins do not change colour when exposed to the air. There are 1-7 yellow brown spores in each spore sac measuring 25-50 x 17-38 μm (excluding ornamentation). The spores are covered with a raised network 2 to 4 μm high.

Figures 12 - 14. The Burgundy truffle (Tuber aestivum = Tuber uncinatum) has a characteristic rough surface and a coffee-coloured interior. The spores are ornamented by a raised network. (Figure 12 Danell & Wedén).
2.1.4 Chinese truffle (*Tuber indicum*), Figures 15 - 17

The Chinese truffle, *Tuber indicum*, is the most important of the Asiatic truffles. The truffles can be 10 cm or more in diameter, have a highly variable appearance and may resemble the Périgord black truffle. The surface ornamentation can be nodulose or covered in pyramidal warts. The skin is 500-700 μm thick (including warts) with an outer layer of a crust of nearly globose, dark reddish black cells and a slightly pigmented inner layer of intricately arranged thin-walled cells. Cells in the inner layer are paler, radially elongated in the outer zone and rounded towards the inner. The contents of truffles, which resemble the Périgord black truffle, are dark purplish black with numerous very thin whitish veins composed of colourless, thin-walled cells, 5-10 μm diameter. The spore sacs contain 1-6 subglobose or elliptical spores that are transparent when young becoming reddish to dark brown at maturity. They measure (15-) 22.5-25 μm x 30-35 μm (including ornamentation). When immature the spores are similar to those of Périgord black truffle but a little smaller, less elliptical, and with fewer and larger widely spaced spines 3-5 μm high and 1-3 μm wide at the base (yellow arrows in Figure 17). As the spores mature the bases of the spines become joined with ridges (pink arrows) forming an incomplete reticulum quite different from spores of the Périgord black truffle.

*Figures 15 - 17. Chinese truffles (Tuber indicum) are not unlike the Périgord black truffle and are two species are easily confused by the novice. However, the two species can be distinguished by their spores with the Chinese truffle having fewer spines (yellow arrows) when young which gradually become joined together with ridges (pink arrows) at maturity.*

2.2 Identification of immature truffles

Problems can be experienced when trying to identify anything other than fresh and mature truffles. For example, the spores inside immature truffles such as those illustrated in Figures 6, 7 and 8 can contain either no spores or spores that have yet to develop any ornamentation (Figures 18 and 19). Molecular techniques are the only way to confirm the identity of these and truffles which have been frozen, defrosted and allowed to decay (Figures 20 and 21).
Figures 18 and 19. Above: a section of an immature truffle which has spore sacs (asci) but no spores inside them. Below: spores inside developing truffles can be without any ornamentation that would help with their identification.
Figures 20 and 21. Two examples of truffles that had been frozen, defrosted and then allowed to decay. Neither had internal nor external structures that could be used to identify them.
3 Relative values of some black truffles

3.1 Tuber brumale (winter truffle)

While the winter truffle has a value and is traded in Europe (MAF Biosecurity) it is a poor relation of the Périgord black truffle. Below are some excerpts quoted verbatim from the Internet comparing the gastronomic and monetary values of the winter truffle (Tuber brumale) and the Périgord black truffle (Tuber melanosporum).

http://www.frenchselections.com/deco/truffle.htm (Joie De Vivre, Frenchselections.com)

Tuber brumale (truffe musquée). This other black truffle grows at the same time and in the same areas as the melano. The black scales of the skin are smaller and the white veins in the black flesh are fewer but thicker. The scales flake off easily when the truffe is brushed. Its taste and aroma are substantially lighter and different from the melano. Price varies greatly from one variety to the next, and also from one year to the next. Typically, the melano may cost 5 times more that the brumale, indicum or aestivum. The magnatum is the most expensive of all, about 4 times more than the melano.

http://www.bctruffles.org/2006%20Fall.pdf (Truffle Association of British Columbia, Fall 2006)

Major concerns to the developing industry:

• Using the right species of tuber for inoculations: Tuber brumale looks very similar to T melanosporum, however is worth 1/5th the value.

• When purchasing to T melanosporum from importers often it is not T melanosporum at all.

• T brumale is very competitive and once in a truffier, or on a trees root system it is nearly impossible to get rid of short of removing the soil and tree from the site.

http://www.finefoodproducts.info/pages/f-truffle.htm (Van Raalten Import, The Netherlands)

The winter truffle, Tuber brumale as it is called. This truffle species, resembles the much higher prized precious black truffle, can be recognized as well by its strong smell and taste, but is significantly less in quality and costs even less than half! We usually trade during wintertime only in Black Precious Truffles from Norcia or Spoleto, and avoid complaints.

http://www.truffoir.go.ro/En/species.htm (Truffoir, Romania and Hungary)

Winter truffle (Tuber brumale Vitt.) Harvested between November and March, on the same sites as Mélano which it strongly rivals. Darker epidermis, flaking off easily. Soil less subtly marbled with a grey background. Smells of turnip, nuancée with garlic (suavum version) ethereal, disagreeable (version moschatu). Taste: Very peppery and characteristically turnipy.

www.viavinum.es/viavinuming/newsletter/0611_noticia_newsletter2_ing.html (Viavinum, The Wine Tour Company of Spain)

In the world there exist more than 70 varieties of truffles, of which more than 30 can be found in the Old Continent, although, without a doubt, the two types of truffles more searched for by their culinary value are the white Italian truffle and the black of Périgord that is typical of
France and Spain and is known by its black colour and its strong and sharp aroma. Its flavour is pleasant, although slightly bitter. Very similar to this last one is the *Tuber brumale* that, although of inferior quality and price, can be collected in the Spanish forests. Finally we should also mention the summer truffle that, on the contrary than the previous ones, grows from summer to the beginning of autumn, has a flavour that remembers the nut and has a price rather more reasonable than most of truffles.


The top four sought-after varieties of fresh truffles are sky-high to begin with. The prices vary widely among varieties (from sky-high to astronomical), and from one year to the next, depending on the size of the harvests. Typically, the *Melanosporum*, the top black truffle, may cost five times more than the *Brumale, Uncinatum* or *Aestivum* – currently $1,000 to $2,100 a pound. The *Magnatum* white truffle is the costliest: It can be four times more expensive than the *Melanosporum*.

http://www.truffle-and-truffe.com/especes-uk.htm (Les produits d'un terroir)

To the nose, the perfume is sometimes agreeable, but often strong and musky. On the palate there is a slight bitterness and taste of humus soil.

### 3.2 *Tuber indicum* (Chinese truffle)

The organoleptic qualities of Asiatic truffles are generally considered to be inferior to those of the Périgord black truffle. This maybe because these truffles are simply inferior or because they are harvested by digging and without the aid of dogs so that immature, mature and over mature are sold together. Whatever the reason the price of Chinese truffle is a small fraction of that commanded by the Périgord black truffle. Typically, Chinese truffles can be purchased and delivered to Europe for less than US$100/kg and sell for less than 10% of this in Yunnan (e.g. Chinesetruffle.com 2008; Spencer & Randall 2006). In contrast, the current price for Périgord black truffles in season ranges from US$1232 in the Aups, France, market, US$1760/kg for North Carolina produce (Lopez 2008), and NZ$3500 (US$2740) for grade 1 New Zealand truffles at the farm gate (truffles produced in the Northern Hemisphere off-season). Clearly there would be some concern if Chinese truffles were to find their way into New Zealand truffières (see section 4.1).
4 The risks of using truffles to produce infected plants

Truffle infected plants can be produced in a number of ways (Hall et al. 2007). While inoculating with spores remains the method of choice for the majority of nurseries there is a danger that the inoculum will be contaminated with other species of truffle. Consequently, some nurseries prepare their inoculum from fresh truffles that have been screened in an attempt to ensure it does not contain truffles of the wrong species. This also gives the nursery the opportunity to prepare inoculum only from large truffles in the hope that like will begat like.

However, no matter how careful a nursery might be there is always a chance that inocula might be contaminated. For example, those nurseries that prepare their inoculum from powdered or sliced and dried truffle (Figure 22) are relying entirely on the skill of the supplier and an accidental or careless inclusion of even a small piece of, for example, *Tuber brumale* or *Tuber indicum*, could spell disaster for a grower. Similarly, tiny pieces of a contaminating truffle, perhaps from a rotting truffle delivered in the same container (Figures 20 and 21), might get trapped inside a small crack in the surface of a truffle (Figures 23).

![Figure 22. Sliced and dried truffle is a convenient inoculum but the inclusion of even a small piece of truffle of the wrong species could ruin a whole batch of inoculated plants.](image-url)

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**Figure 22.** Sliced and dried truffle is a convenient inoculum but the inclusion of even a small piece of truffle of the wrong species could ruin a whole batch of inoculated plants.
This small piece of contaminating truffle could easily go undetected because the piece sampled to confirm the identity of the truffle did not include the contaminating piece, for example, it was taken from the wrong side of the truffle. Similarly, if the contaminant consisted of just a few spores measuring only 0.02 mm (20 μm) wide it could escape the detection of even the most sensitive molecular techniques. Consequently, the revised New Zealand MAF Import Health Standards (2007) will not prevent more *Tuber brumale* entering New Zealand nor the entry of another unwanted species such as the Chinese truffle (*Tuber indicum*).
4.1 Control of contamination on truffle plants in nurseries

In Europe *Tuber brumale* and the Chinese truffle command a much lower price than the Périgord black truffle (section 3) and perhaps because of this *T. brumale* is regularly found contaminating commercially produced plants in Europe. Over the past decade the Chinese truffle has also been allowed to enter European truffières (Kington 2008; Milius 2008; Murat et al. 2008).

Some nurseries try to safeguard the quality of their plants by carefully inspecting the mycorrhizas prior to sale. Although those of the Périgord black truffle are somewhat different from the winter truffle (Figures 24 and 25) the roots must be sampled at the right time of year to see the diagnostic characteristics. However, because not every mycorrhizal root tip on every plant can be screened there is still a danger that plants can be contaminated - a problem that even molecular techniques cannot circumvent.

Figure 24. *Tuber melanosporum* (left) mycorrhizas have long, branched projections from the surface of the mantle (cystidia) whereas *Tuber brumale* (right) mycorrhizas has unmistakable short, stout, needle-like projections.

Figure 25. The surface of *Tuber melanosporum* (left) and *Tuber brumale* (right) mycorrhizas look like crazy paving but *T. brumale* has smaller, more deeply lobed cells.
Stringent requirements on the quality standards for truffle inoculated plants distributed in New Zealand would also help limit the chance of *Tuber brumale* (and Asiatic truffle) spreading. Regulations for this are already in place in parts of Europe (Hall et al. 2007) and might be considered for New Zealand to augment the Fair Trading Act (1986) and the Consumer Guarantees Act (1993).

The New Zealand Truffle Association needs to consider whether the onus should lie on the producer of truffle infected trees to screen their plants against contamination or whether, for example, the purchaser of truffle infected trees would be best to get plants assessed by an independent specialist. It is also important for potential growers to be aware of what methods are required to distinguish infections by the Périgord black truffle and *Tuber brumale* and the true meaning of phrases such as:

- Inoculated with the Périgord black truffle,
- Infected with the Périgord black truffle,
- Mycorrhizas with features consistent with black truffle,
- Solely infected with the Périgord black truffle with x% of the root tips showing mycorrhizas

### 4.2 The third line of defence

If all else fails and a contaminating truffle escapes detection when the truffles are being checked prior to the inoculation process or inspection before a plant leaves the nursery, it is still possible to detect it after the trees are outplanted. This might be done by randomly sampling the roots of plants in a truffière annually and in midsummer when the cystidia and patterns on the mantles are easiest to see under a microscope (Figures 24 and 25). Molecular methods using species specific primers, on the other hand, could be used at any time of the year and might also be made quantitative (see Hall et al. 2007 page 95; Hall et al. 2008; Linnaeus Ltd 2008).

If a contaminating truffle is detected in a truffière, is detected soon enough say before it has spread to other plants, and its eradication is warranted, then contaminated trees could be removed roots and all and burnt, and the soil treated with a fumigant such as methyl bromide, methyl isothiocyanate or Basamid granules.
5 Some useful references


Linnaeus Ltd. @008. www.linnaeus.co.nz


6 Acknowledgements

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