Truffles and Mushrooms

(Consulting Ltd)

Minimising the risks - some advice for hopeful truffle growers

Ian Hall, Alessandra Zambonelli
& Patricia Nelson

Revised
July 2015

P.O. Box 268, Dunedin, New Zealand
telephone +64-3-454 3574
mobile: +64-27-226 1844
email: info@trufflesandmushrooms.co.nz
web www.trufflesandmushrooms.co.nz

Contact details for the authors:

Dr Ian R. Hall, Truffles & Mushrooms (Consulting) Ltd, P.O. Box 268, Dunedin 9054, New Zealand. Telephone +64-3-454 3574, +64-27-226 1844, truffle1@ihug.co.nz, www.trufflesandmushrooms.co.nz

Dr Alessandra Zambonelli, Dipartimento di Scienze Agrarie, Via Fanin 46, I 40127 Bologna, ITALY, zambonel@agrsci.unibo.it

Mrs Patricia Nelson, 8 Upland Road, Remuera, Auckland, New Zealand, gpetal@ihug.co.nz

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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 (Tuber melanosporum) and winter truffles (Tuber 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.

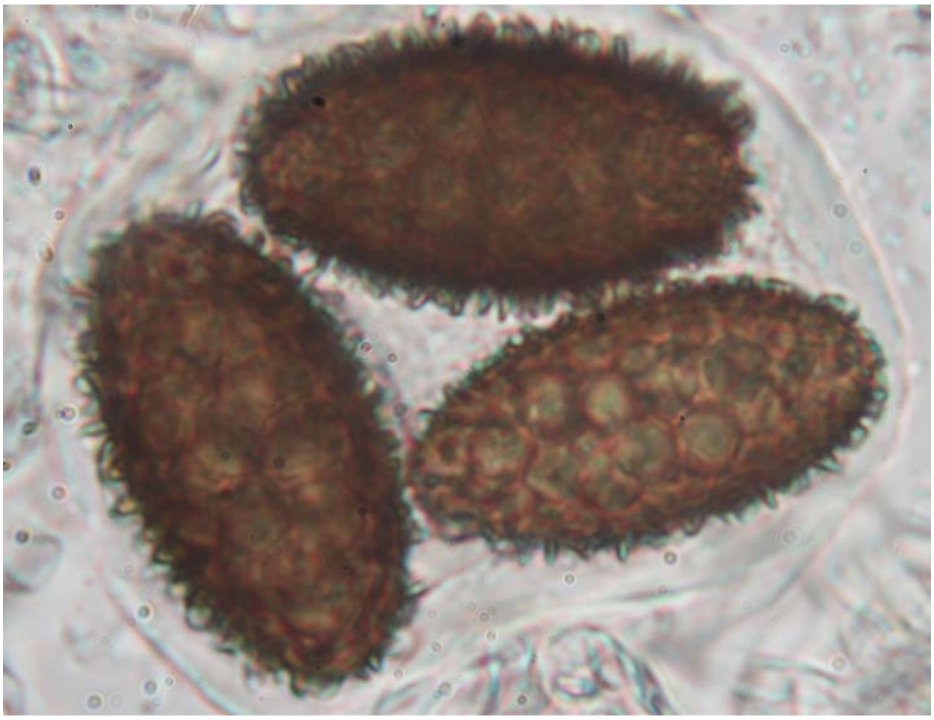
Figure 5. Four species of black truffles that might be confused by a novice. Clockwise from top left: Périgord black truffle (Tuber melanosporum), Chinese truffle (Tuber indicum), winter truffle (Tuber brumale) and Burgundy truffle (Tuber aestivum). Compare the broad white lines in the T. brumale truffles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

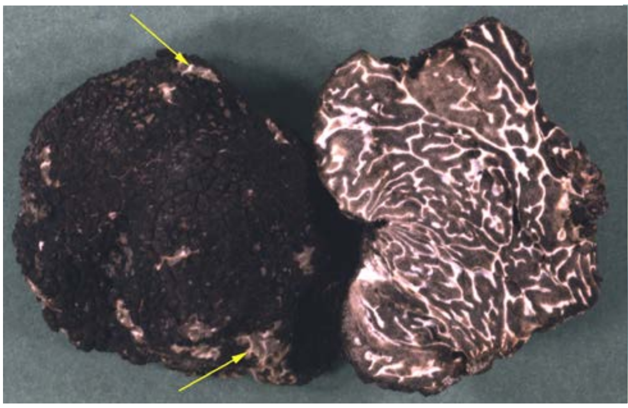
Figures 6-8. Young, immature Périgord black truffles have whitish contents. From top left: truffles harvested in early March 1997 in Alan and Lynley Hall's truffle, Gisborne, New Zealand; immature, rejected truffles in Pébeyre's establishment in Cahor, France, some showing where a small slice has been removed to reveal the contents of the truffle; and immature frozen truffles imported into New Zealand during the Northern Hemisphere off season.

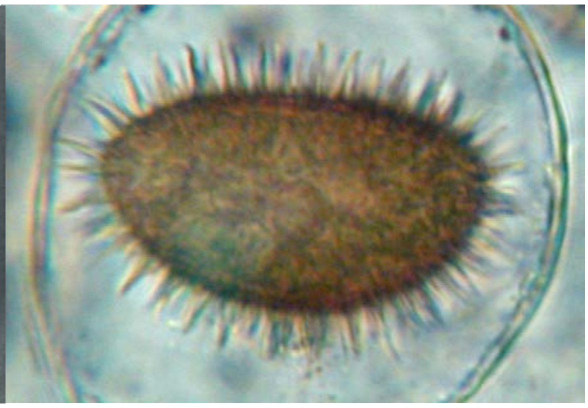
Figure 9. Spores of the Périgord black truffle are covered with short spines which are usually curved at their tips. There can be 1 to 6 spores in each spore sac (ascus) which is the clear, greyish circle surrounding all three spores.



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).

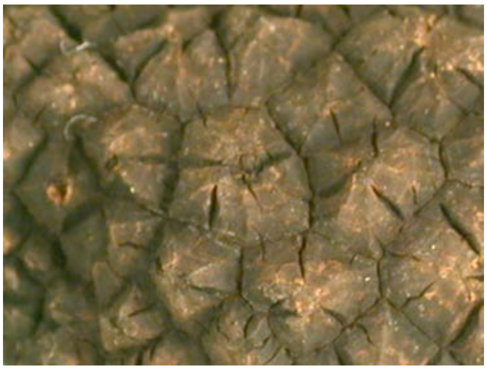




Figures 10 and 11. Winter truffles (Tuber brumale) showing the fragile skin that is easily damaged (yellow arrows), the wide veins criss-crossing a greyish brown interior, and a spore ornamented with the characteristic long, straight spines.

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

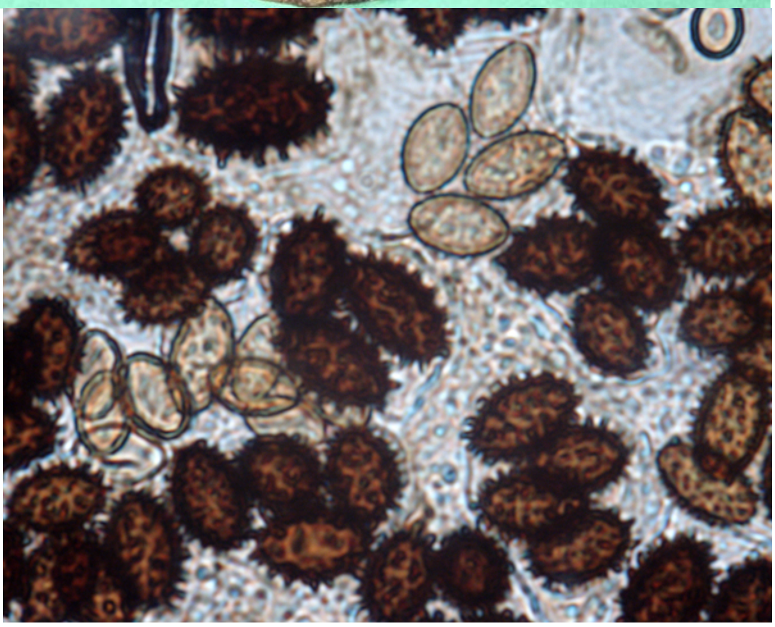
Burgundy truffles are brown to black and 2 cm to more than 10 cm in diameter. The skin adheres to the tissues beneath and is ornamented with 5-7 sided pyramidal warts 3-9 mm wide, with longitudinal fissures and some fine transverse markings. When mature the inside of the truffle is a dark brown. The 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.

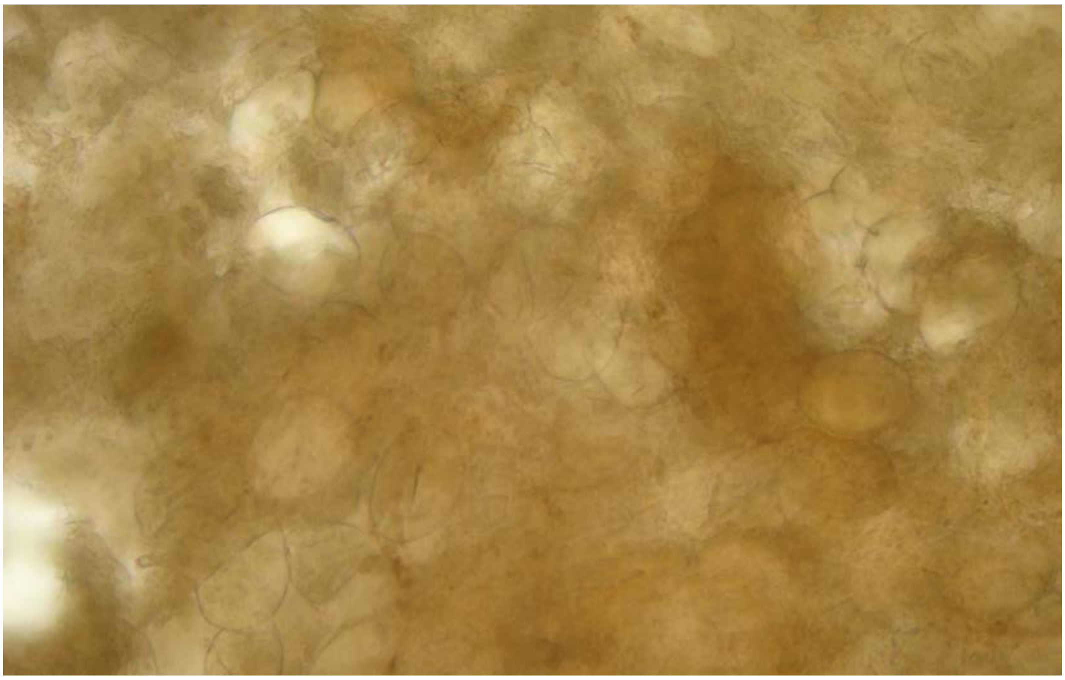


Figures 15 - 17. Chinese truffles (Tuber indicum) are not unlike the Périgord black truffle and are two species are easily confused by the novice. However, the two species can be distinguished by their spores with the Chinese truffle having fewer spines when young which become joined together with ridges at maturity (Chen et al. 2011).

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 identify 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. Note the nematode inside the ascus on the left-hand side of the photo below (red arrow)

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 past 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.hts (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."

http://www.thenibble.com/reviews/main/vegetables/truffle-glossary.asp?r=rss (The Nibble, Great Food Finds)

"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 700 Yuan (e.g. Chinesetruffle.com 2008; Spencer & Randall 2006; Hall unpublished data). In contrast, the current price for Périgord black truffles in season ranges from around US$1000 to NZ$3500 (US$2300) for grade 1 New Zealand truffles at the farm gate and produced counter-season to the Northern Hemisphere. Clearly there would be some concern if Chinese truffles were to find their way into New Zealand truffières (see section 4.1). Currently several hundred tonnes of *Tuber indicum* are harvested annually.

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 inocula from fresh truffles that have been screened in an attempt to ensure they do not contain truffles of the wrong species. This also gives nurseries the opportunity to prepare inocula only from large truffles in the hope that like will begat like.

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 supplier not to accidentally include a small piece of, for example, *Tuber brumale* or *Tuber indicum*. Similarly, tiny pieces of a contaminating truffle (Figures 20 and 21), might get trapped inside a small crack in the surface of the truffle of choice (Figure 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.

A small piece of contaminating truffle measuring only 1 mm^3 could easily go undetected particularly if it was hidden in a small fissure on the surface of a truffle (Figures 3 and 23) particularly if the sample of a truffle was taken from the opposite side to the fissure. Some nurseries might disregard such a possibility but a 1 mm^3 piece of truffle could contain 50,000 spores – sufficient to contaminate maybe 100 trees under ideal conditions.

Figure 23. The tiny 1 mm^3 piece of truffle in the red square could hold enough Tuber brumale or Tuber indicum spores to contaminate about 100 plants grown under ideal conditions. This small piece could easily go unnoticed and miss being washed off if it was tucked into one of the cracks on the surface.

Another possibility that needs to be considered is the natural variability in truffle spores. Clearly, distinguishing a *T. melanosporum* spore like the one illustrated in Figure 9 from a *T. brumale* spore illustrated in Figure 10 poses no problem. However, what is the one shown in Figure 24? Is this an anomalous *T. brumale* spore or a *T. melanosporum* spore?

Figure 24. Spores from a truffle that had all the features of Tuber melanosporum but have spines somewhat different from those in Figure 9.

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 and in some parts of the world this is required by law (Regione Emilia-Romagna 2013). Although those of the Périgord black truffle are somewhat different from the winter truffle (Figures 25 and 26) the roots are best sampled when the fungus and roots are growing quickly – typically during late summer – so that the diagnostic characteristics can be easily seen. 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.

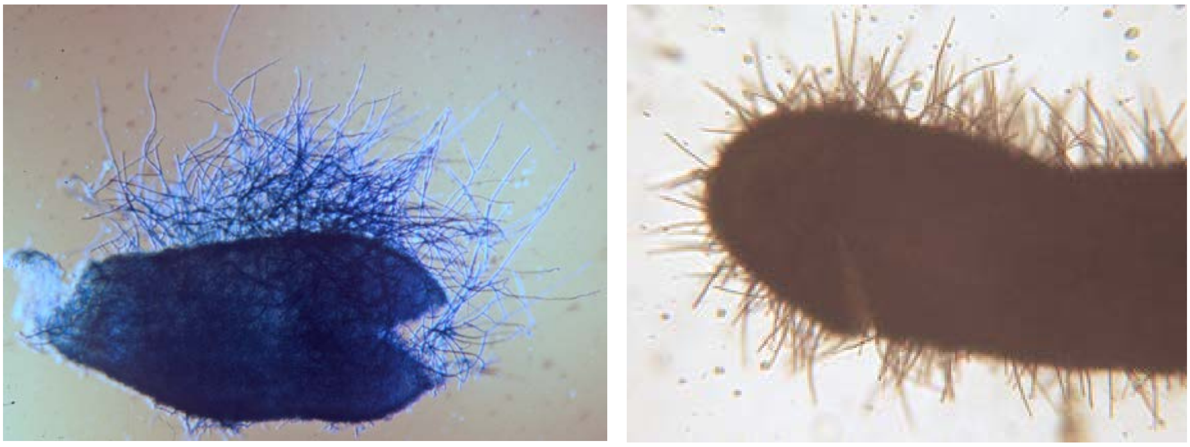


Figure 25. Tuber melanosporum (left) mycorrhizas have long, branched projections from the surface of the mantle (cystidia) whereas Tuber brumale (right) mycorrhizas has unmistakable short, stout, needle-like projections.

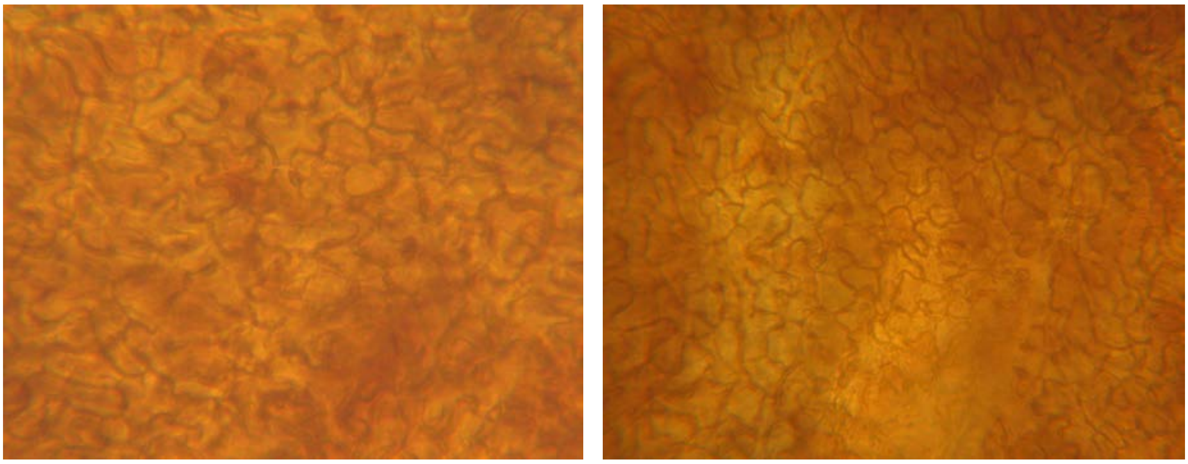


Figure 26. The surface of Tuber melanosporum (left) and Tuber brumale (right) mycorrhizas look like crazy paving but T. brumale has more deeply lobed cells (the two photos are not to scale – T. brumale cells are larger).

Stringent requirements on the quality standards for truffle inoculated plants distributed in New Zealand would also help limit the chance of Tuber brumale, Asiatic truffle, and other non-truffle contaminants such as AD spreading. Regulations for this are already in place in parts of Europe (Hall et al. 2007; Regione Emilia-Romagna 2012) and might be considered for New Zealand to augment the Fair Trading Act (1986) and the Consumer Guarantees Act (1993).

Countries which do not have legislation in place need to consider whether the onus should lie on the producer of truffle infected trees and for them 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 such as “dnature” (www.dnature.co.nz).

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 slips by the inspection process 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 can be quantitative (see Hall et al. 2007 page 95; Hall et al. 2008; dnature 2015).

If a contaminating mycorrhizal fungus, whether it be another species of truffle or something like AD, and is detected before it has had time to 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 How growers can protect themselves

It is important that potential growers become conversant with all aspects of truffle growing before they begin to enter the industry. To help with this they can read one of the many books that are available in a number of languages. For example, Taming the Truffle (Hall et al. 2007) was written in plain English specifically with the grower in mind. The newer book by Mocillo et al. (2015) has a broader scope but does a similar job. Then there are numerous truffle growers' associations around the world that can provide assistance some of which have regular publications to keep their members up to date.

5.1 Uninfected trees

Nurseries can sometimes have failures and produce trees that are completely uninfected. Some possible reasons for this are:

- Failure of the truffle to infect,
- Greenhouse conditions unsuited to the truffle fungus,
- Failure to follow proven methods.

It is also possible that after outplanting, truffle infections could simply be lost because the conditions in the field might be totally unsuited to the fungus. With care this might have been avoided by carrying out a full suite of soil tests and making sure that the climatic conditions fell with the boundaries.

5.2 Contaminated trees

Mycorrhizal fungi other the truffle of choice can often be found on trees in truffières. Sometimes the origin of this contamination can be traced back to the nursery where it may be linked to:

- The use of truffles that were contaminated with another species of truffle (see above),
- Airborne fungal spores that enter a greenhouse via windows and doors,
- Fungi resident in the nursery such as AD (Figure 27).

Contamination after planting can also occur. Possible sources include:

- Machinery used to work the land,
- Contaminants on boots and on the feet of animals,
- Already present in the soil at the site,
- From surrounding ectomycorrhizal trees.

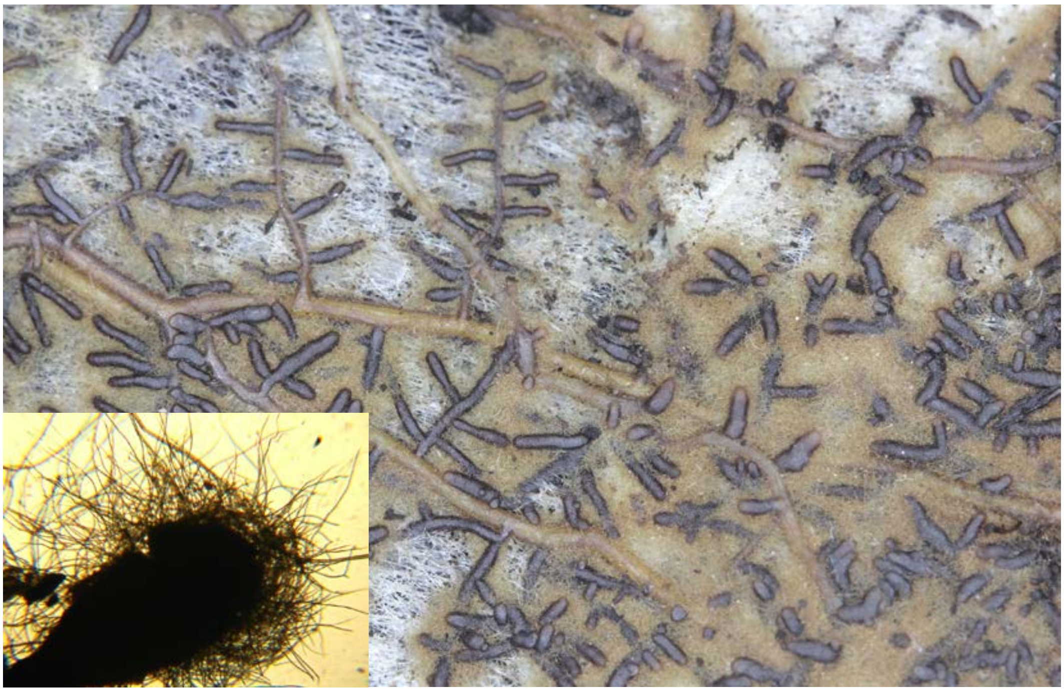


Figure 27. The AD fungus (Trichophaea woolhopeia), a highly competitive fungus that can rapidly colonise truffle plants while they are still in the greenhouse.

5.3 Effect of fertilisers

Fertilisers have been used to fertilise truffières as far back as the 1970s in the hope of encouraging growth of the truffle fungus. French examples include Fructitfuf (Pebeyre et al. 1985), Lombrisol, Phaligal and Vegethumus. The effect of these were summarised by Bradshaw in his PhD thesis (2005):

> Fertiliser application is not often recommended in commercial truffières in France and elsewhere in Europe as varied results have been observed (G. Chevalier pers. comm.). Furthermore, the composition of fertilisers that have been used in France, for example, is often not available with the exception to acknowledge their organic or inorganic status. Four products that have been examined experimentally include Fructitruf (1150 kg ha-1, 6 % organic N, rich in K & Mg), Phaligal (530 kg ha-1, organic, composition unknown), Vegethumus (530 kg ha-1, composted sheep manure) and Lombrisol (530 kg ha-1, worm castings/compost) (Verlhac et al. 1989). The application of these products to a producing truffière (established in 1972) was examined over four seasons and results were variable. In each case, fertiliser application reduced the number and weight of truffles from producing trees. This was most notable in those trees that were treated with Fructitruf. The number of producing trees increased slightly when treated with Vegethumus and Lombrisol but this may have been an artefact of truffière age as all treatments (including the unfertilised control) showed an increase in the number of producing trees over the experimental period (Verlhac et al. 1989). Ongoing results of this and other similar experiments are not documented and similarly, experiments utilizing inorganic fertilisers are lacking in the literature. In New Zealand and Tasmania, the effect of fertiliser application has not been published in any form with the exception of a glasshouse trial by Brown (1998). This study found the levels of infection of T. melanosporum were maintained at applied P rates as high as 150 mg kg-l soil (-250 kg P ha-1) and the point was argued that P application could be used to limit the development of native Australian ECM

> fungi given their low tolerance to elevated soil P. The P-fixing ability of the soil used in the experiment (a grey sandy loam) was not reported. There was no determination of the impact of applied P on ascocarp production of *T. melanosporum* in the field.

Gerard Chevalier (1998) was more direct:

> The fertilization must be carried out according to soil analysis. The truffle growers can use mineral and/or organic fertilizers (CHEVALIER and POITOU, 1990). The only organic fertilizer used is the Fructitruf. The opinions about this product differ very much (OLIVIER et al., 1996). Anyway it is sure that it is better not to fertilize than fertilize badly. An unsuitable fertilization can sterilize a truffière.

It is also known that a lack of phosphorus can increase the growth of mycorrhizal fungi in the soil (Wallander & Nylund 1992), the application of phosphorus and nitrogen can markedly reduce mycorrhizal formation (Smith & Read 2008) but may also interact (Hall et al. 1984). For example, Suz et al. (2010) found that when a high phosphorus fertilizer was applied to foliage, *T. melanosporum* root colonization increased, whereas when the same fertilizer was applied to the soil, indigenous competitive fungi were enhanced at the expense of *T. melanosporum*. Furthermore, in a meta-analysis across all mycorrhizal studies, mycorrhizal abundance decreased 15% when plants were fertilised with nitrogen and 32% with phosphorus fertilisation (Treseder 2004).

Perhaps the most telling observation about soil nutrients and truffle production comes from a tiny truffière planted in 1988. The soil has an Olsen extractable phosphorus concentration of just 2 (very low) and a carbon/nitrogen ratio of 13 (i.e. deficient in nitrogen), but last year produced nearly a kilogram of truffles per tree. And finally the most scrawny, poor growing trees that seem to be struggling to survive often produce the most truffles in a truffière.

5.4 *Minimising the risks*

Here are some suggestions on how a future grower might help protect themselves:

- Have your soil and site fully appraised before you do anything.
- Have trees independently tested before purchase.
- Don’t use untested methods/products in your truffière.
- Question a supply contract if it is loosely worded, for example:
    - Inoculated with the Périgord black truffle = the fungus has only been put on the tree roots – there may be nothing actually growing there,
    - Infected with the Périgord black truffle = the fungus is growing on the roots but no mention is made of whether other mycorrhizal fungi are present,
    - Mycorrhizas with features consistent with those produced by *Tuber* = could be any species of *Tuber*,
    - Using the technique recommended by, developed by, advice given by, etc. - maybe trying to pass on responsibility to a third party,
    - Solely mycorrhized with *Tuber melanosporum* on the roots with x% of the root tips showing mycorrhizas = only the Périgord black truffle is on the roots.

6 Some useful references

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7 Acknowledgements

We wish to thank Chris Pratt for helpful comments during the early phase of preparing this document and Eric Danell and Christina Wedén for Figure 12.