



Mycorrhiza testing of truffle trees

Courier or drop-in samples to:

Alexis Guerin, Mycotree, C/-Southern Woods Nursery, 1002 Robinsons Road, Templeton, Christchurch New Zealand.

Please contact me if you have any questions: alexis@mycotree.co.nz | 027 434 0387

Why should I test mycorrhizae¹ of my truffle trees?



Mycorrhizae of the Black truffle in the field on Quercus robur (left) or on Q. ilex (right)

1. It is doable and relatively affordable.
2. Microscopy can detect the level of development of truffle mycorrhizae which, in turn, indicates the fruiting potential.
3. There is no point spending time/efforts on tree management if the target truffle mycorrhizae cannot be found on the roots.

How many trees should I sample?

It depends on the extent of information you wish to get and its statistical interpretation.

The more trees you sample, the better but 5% of trees is a minimum and a good compromise between a reasonable sample size (from a cost point of view) and robust information.

A first sampling provides result trends that can be further tested/comforted via a more educated second testing round.

About mycorrhiza testing at Mycotree:

Alexis Guerin-Laguet Ph.D. has over 27 years of experience in the cultivation of edible mycorrhizal fungi with strong academic publications track records (www.mycotree.co.nz).

I identify Périgord Black (*Tuber melanosporum*) and Burgundy (*T. aestivum*) truffle mycorrhizae at the species level using a combination of dissecting and compound microscopy analyses. I also

¹ The truffle/mushroom fungi colonize roots and transform them into **mycorrhizae** (from Greek, literally means 'fungus-root'), real root organs resulting from the intimate merger between plant and fungal tissues. A great nature story!

identify Bianchetto truffle (*Tuber borchii*) mycorrhizae by microscopy but DNA analysis through a contractor is compulsory to confirm their identity.

DNA technology is the most objective and sensitive method to identify all micro-organisms, including edible mycorrhizal fungi (truffles or mushrooms). However, only microscopy offers a real-life assessment of the abundance of fresh and active mycorrhizae of your target species in root samples. Therefore, combined microscopy and DNA testing offer the best of all analyses. There are also situations where microscopy alone can be as objective as DNA: in New Zealand conditions, mycorrhizae of the Périgord Black and Burgundy truffle can be identified by microscopy only, without DNA testing, so long as all key morphological characteristics of these two types of mycorrhizae are present. This is not the same for Bianchetto truffle whose mycorrhizae resemble those of many other truffle species present in New Zealand. DNA testing is therefore compulsory in conjunction with microscopy to identify *T. borchii* mycorrhizae. Similarly, *T. brumale* mycorrhizae can only be identified using both microscopy and molecular analyses. In any case, I will provide the best mix of microscopy and DNA data for your needs.

Our service focuses on the **detection** of mycorrhizae of your target truffle species, and **an estimation of their abundance/development**, which is the most important information for you.

I also comment on **the presence of mycorrhizal fungi other than your target truffle species** in the root samples. However, for accurate identification of non-truffle species, DNA testing is compulsory (I would advise on a case-by-case basis).

Cost:

Microscopy

Target species, detection, and abundance: \$42 per root sample, i.e., per tree sampled.

If you wish me to collect root samples for you, please count an additional sampling charge of \$100/h excl. GST and mileage expenses (\$1.20 per km).

DNA

I would let you know if DNA testing were necessary to confirm target species (for *T. borchii*, DNA testing always is).

For *T. melanosporum*, please note that a **mating type test** can also be organised for any positive tree to identify the mating type(s) present in the corresponding sample.

I need an additional ≈10 minutes per sample to select/collect mycorrhizal tips for DNA testing.

Cost TBA.

Report fee

\$200/h excl. GST

The report is important to summarise/explain results and provide recommendations. The fee depends on how many root samples you send. It is difficult to estimate the time required as it depends also on results, but as an indication, I may need ≈1-2h for 10 samples.

Please see the root sampling protocol and examples of samples on the following pages (3 to 7).

Root sampling protocol

1. Dig the soil carefully with a hand hoe and fork (e. g. cuttlefish hoe) and look for roots by starting at the surface and slowly clearing away soil by hand (within 15 cm depth). You may start just outside the drip line of the tree (or at the edge of the brûlé if any). However, if no luck (especially under *Quercus ilex*), just try another patch, even close to the trunk since this may help finding the tree roots. Look for woody root pieces bearing fine roots (grass roots are thinner, usually less than 1 mm wide, whitish, and usually close to the surface). Good pieces are 10-15 cm long, but not too thick say 1-5 mm in diameter. Make sure that the pieces collected bear fine roots where the mycorrhizas are. As required, repeat the operation on another side of the tree. Sampling from different sides will increase the chance to detect your target truffle species. You can mix roots from different sides (i. e. just one root sample per tree). **Please provide between five to ten root pieces (each 10-15 cm long) per sample (i. e. per tree).**
2. Collect root pieces carefully (by cutting one or both ends, as required), taking care to 'dig' the root piece out rather than 'pulling' it out to avoid tearing off mycorrhizae. Please do not wash the roots but leave attached soil on. Soil lumps attached to the roots may hold mycorrhizae, so it is better not to disrupt them. Wrap roots with attached soil in a moist white serviette or kitchen handy towel (paper hand towels, e.g., Tork, are great) and put them in a compostable resealable bag. Please use only water (tap water is fine) to moisten the serviette.

Please have a look at the attached photos (pages 4-5) to make sure you send a root sample of an appropriate size and bearing fine roots where the mycorrhizae are.

***Quercus ilex* are great truffle hosts but can be difficult to root sample! Please see separate example of ilex root samples (pages 6-7).**

3. Record the sampled tree and write its name/coordinates with a permanent marker on the bag. Please also label the sampled tree or record its coordinates in the truffière.
4. Courier/drop the samples to:

Alexis Guerin, Mycotree, C/-Southern Woods Nursery, 1002 Robinsons Road, RD8, Christchurch 7678

This is a rural delivery, but root samples preserve very well so please don't worry about a slight delay in delivery if they are sent late in the week.

Alternatively, you can use:

Alexis Guerin, Academy Hire, 846 Jones Road, Rolleston 7614 (with NZ Courier, Aramex, or Post Haste)

Please let me know where you send to and when since I will need to pick up from there.

Size of the root samples

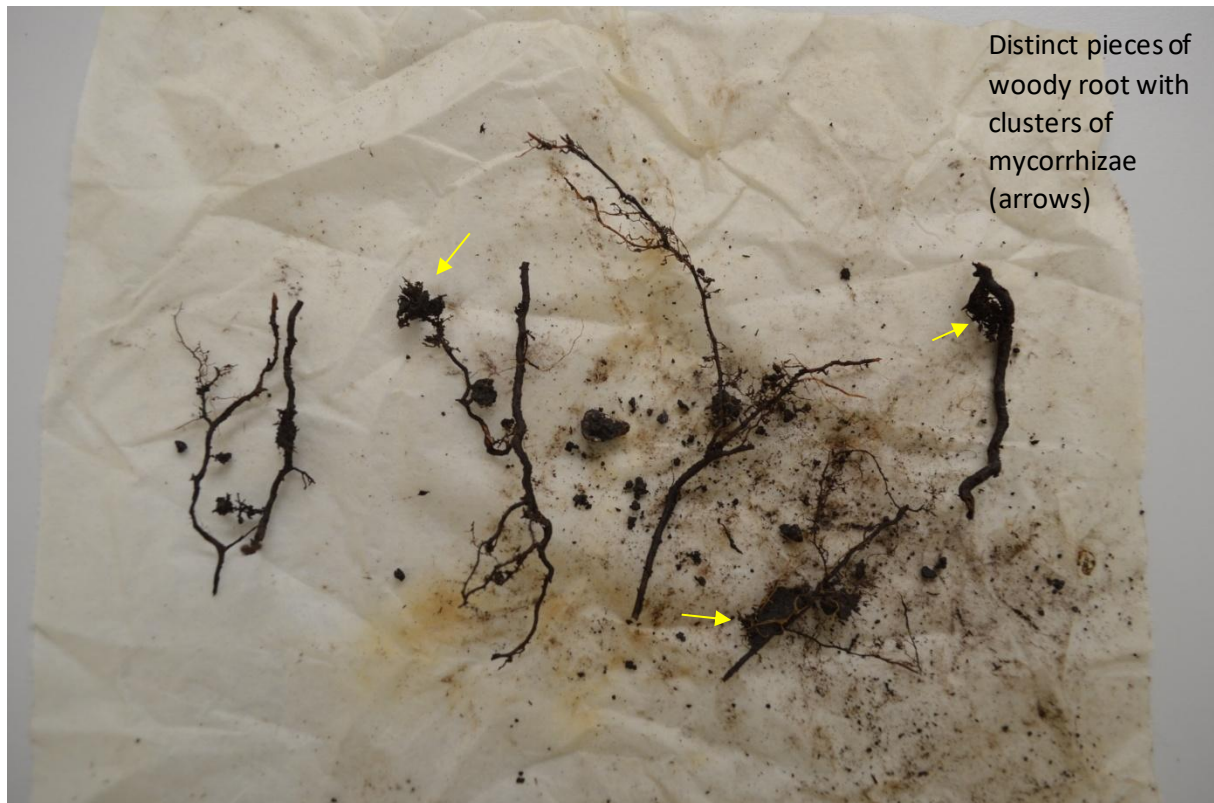
To avoid any size problems (some of the samples we receive are too small or do not bear enough fine roots to carry out analyses) we give here examples of appropriate sample sizes:



A similar one:



The sample below is smaller (10 cm-long pieces) but still appropriate given that several clusters of mycorrhizae are clearly present.



Finally see below more examples of good samples





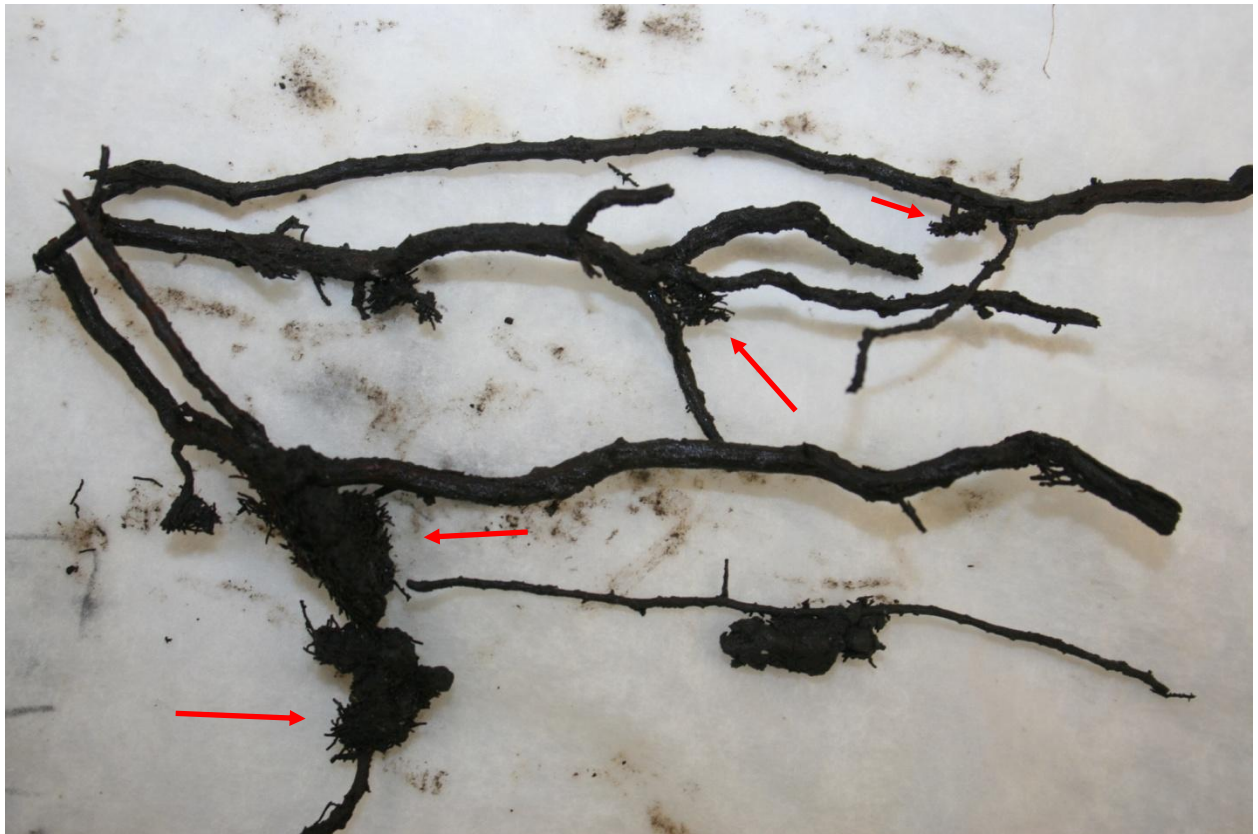
Examples of root samples from *Quercus ilex*



This sample is not ideal but may be OK as there are mycorrhizae to look at. This is the absolute minimum I can work from.



Another ilex sample: still not the best sample but there are more lateral short roots attached. The truffle mycorrhizae we are looking for are potentially on these short lateral roots.



Another sample from ilex with large (several mm wide) clusters of mycorrhizal roots. You can see these aggregates are not just mere lumps of soil but rather soil adhering to short mycorrhizal root tips that you may see coming out of the root/soil aggregates (arrows). If you can find root pieces with such large mycorrhizal clusters attached, it is better than the samples shown above.