# Testing of truffle or mushroom seedlings

*Courier or drop-in seedlings to:*
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## Why testing seedlings?

There is no point planting a seedling to establish a truffle or mushroom orchard if the seedling is not well-mycorrhized by the target species.

Mycotree offers the testing and certification of seedlings inoculated with the following "target" truffle or mushroom species:

- *Tuber melanosporum* (Périgord black truffle)
- *Tuber borchii* (Bianchetto truffle)
- *Tuber aestivum* syn. *T. uncinatum* (Summer truffle or Burgundy truffle)
- *Lactarius deliciosus* (saffron milk cap)

Mycotree's approach to testing is unique, but similar to that used by scientists who test the truffle seedlings of France' biggest commercial truffle nurseries (AgriTruffe and Robin Pépinières) since 1973 (Andrés-Alpuente et al. 2014).

The testing methodology is not destructive and tested seedlings can be returned to you.

## Mycotree offers to:

(1) Check inoculated seedlings to establish if target mycorrhizae are present and verify abundance.

(2) Document the presence and abundance of non-target mycorrhizae.

(3) Detect the presence of non-target truffle mycorrhizae (identify by DNA at extra cost).

(4) Determine the viability of the seedling,

Estimate the proportion of positively identified seedlings[1] in relation to the batch (see below). Only the individual testing of each seedling determines the actual proportion of such seedlings in each batch.

[1] A seedling that is deemed suitable to establish a truffle or mushroom orchard based on the results of points 1 to 4.

## Methodology of mycorrhiza testing

The testing is based on a double-step (dissecting and compound) microscopal analysis completed by DNA testing if required. The double-step microscopy is crucial to confirm the identity of the target species and must be performed on one seedling of each batch. If results appear constant (the morphology of mycorrhizae stays the same), the remaining seedlings in the sample may be tested by dissecting microscope only.

Under New Zealand conditions, the identification of mycorrhizae of *T. melanosporum* and *T. aestivum* by morphology only (i. e. double-step microscopy) is reliable, while mycorrhizae of *T. borchii* must be confirmed by DNA analyses in addition to microscopal analyses (Guerin-Laguette et al. 2013, 2021).

Besides their identification, the abundance of target mycorrhizae is assessed based on their frequence of detection and the presence of branched/young clusters of mycorrhizae (this assessment is made possible by the experience of the observer).

## Testing regime

A batch is defined as a number of seedlings of the same tree species, same seed source, same age, inoculated at the same time by the same staff, using the same method with the same set of spores and grown under the same conditions.

Mycotree recommends testing a random sample of at least 5% of the seedlings. For batches over 500 seedlings, 2% might be enough (depending if the success rate is 80% or over).

Mycotree can travel to collect a random sample of seedlings. Alternative non-biased sampling options are to be arranged if seedlings are couriered to Mycotree. Certified seedlings will be returned at the nursery's expenses.

## Cost

**Testing of a sample of seedlings (2 to 5% in batch):**

First seedling of each distinct batch: $34.50 for a two-step microscopy.

Remaining seedlings in the sample from the same batch:

$17.25 per seedling for a single-step microscopy provided that the mycorrhizae of the seedlings stay similar to that observed on the first seedling. If a new or unusual mycorrhiza morphology is detected, a compound microscope step is again included at the cost of a two-step microscopy.

**Rapid individual testing of <u>all</u> seedlings (only from batches previously tested as described above) is possible and can be arranged @ $110/h.**

Prices exclude DNA testing ($95 per mycorrhiza sample) and disbursement (e.g. mileage).

## Results

Mycotree will provide a concise report advising:

- Presence and abundance of target and/or non target species and their development

- Estimated percentage of seedlings per batch deemed suitable to establish a truffle plantation.

- Overall recommendations for each batch tested and if seedlings of a given batch need further testing.

## Certificates

Batches:

Mycotree's batch certificate implies that the nursery and their customers acknowledge that the results are based on a sample of seedlings.

Only individually tested seedlings that passed the test will be certified and labelled.

## References


Andrés-Alpuente A, Sánchez S, Martín M, Aguirre ÁJ, Barriuso JJ. 2014. Comparative analysis of different methods for evaluating quality of *Quercus ilex* seedlings inoculated with *Tuber melanosporum*. *Mycorrhiza*, 24 (Suppl 1), S29–S37. https://doi.org/10.1007/s00572-014-0563-x

Guerin-Laguette A. 2021. The sustainable cultivation of edible mycorrhizal fungi - furthering the dream. *Mycoscience* 62, 10–28. https://doi.org/10.47371/mycosci.2020.11.007

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https://www.jstage.jst.go.jp/article/mycosci/62/1/62_MYC520/_article

Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y. 2013. Mycorrhiza analyses in New Zealand truffières reveal frequent but variable persistence of *Tuber melanosporum* in co-existence with other truffle species. *Mycorrhiza,* 23, 87–98. https://doi.org/10.1007/s00572-012-0450-2