



## Testing of truffle or mushroom seedlings

Courier or drop-in seedlings 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](mailto:alexis@mycotree.co.nz) | 027 434 0387

### 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<sup>1</sup> in relation to the batch (see below). Only the individual testing of each seedling determines the actual proportion of such seedlings in each batch.

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<sup>1</sup> 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) microscopical 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 two-three seedlings 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 microscopical analyses (Guerin-Laguette et al. 2013, 2021).

Besides their identification, the abundance of target mycorrhizae is assessed based on their frequency 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 a group of seedlings of the same tree species, same seed source, same age, inoculated at the same time and place by the same staff, using the same materials and method with the same source of inoculum and grown under the same conditions.

Depending on the batch size, Mycotree recommends testing a random sample of 2-5% of seedlings.

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):

\$34.50 per seedling for a two-step microscopy. We recommend testing this way at least two-three seedlings of each distinct batch.

Remaining seedlings in the sample from the same batch:

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

**Rapid individual testing of all 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 AJ, 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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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>