PROPOSAL FOR A METHOD OF QUALITY CONTROL AND CERTIFICATION OF PLANTS MYCORRHIZED WITH *TUBER* GENUS FUNGI

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Truffle cultivation in Italy has improved appreciably in recent years subsequent to the use of certified mycorrhized plants,

The method of quality control and certification was introduced in Italy in 1987 and was modified in 1995 and in 2002 to make it more streamlined, rapid, and precise.

The modifications are the result of 10 years of quality control and certification carried out on 12,000 samples of plants taken from 7 nurseries in special agreement with the Department of Applied Biology, Perugia University.

The quality control can only be carried out by highly qualified personnel.

The method consists of:

the evaluation of a mycorrhized plant; on a morphological and biomolecular basis, by means of visual estimation, or the count of root tips subdivided into: bare tips, those mycorrhized by truffle, and other fungi,

The evaluation of a homogeneous lot of mycorrhized plants.

The criteria for the evaluation of a mycorrhized plant and a mycorrhized plant lot.

A – ANALYSIS OF A MYCORRHIZED PLANT DESTINED FOR TRUFFLE CULTIVATION

A1 – Evaluation of the degree of mycorrhization of a plant by means of visual estimation.

The extent of mycorrhization of a plant is determined with the following operations:

Remove the plant from the container and gently wash the root apparatus taking care to reduce to a minimum the loss of mycorrhized tips by detachment;

Examine by stereo microscope the entire root apparatus for the purpose of identifying the presence of mycorrhizae attributable to the Tuber genus and other possible forms that can be differentiated by morphological character.

Identify the mycorrhizae ascribable to the Tuber genus by means of observation with an optical microscope.

Estimate the percentage of tips mycorrhized by truffle, those blatantly and those mycorrhized by other fungi, by carefully observing the entire root apparatus.

Sample 10 mycorrhizae morphologically ascribable to the *Tuber* genus to be put under biomolecular examination.

When the estimated values do not allow declaration with absolute certainty of a plant suitable or not for the purpose of truffle cultivation, or in cases of doubt, proceed to the examination of the root tips.

COMMENT A1 – The distribution of the mycorrhizae in a root apparatus is generally not homogeneous therefore the percentage of tips mycorrhized by truffle and/or other fungi can be noted only through meticulous observation of all of the roots by using a stereoscopic microscope.

This observation allows the detection of even single foreign mycorrhizae which go undetected when observing only fragments of root apparatus and also allows faster analysis and estimation of the percentage of tips mycorrhized by truffle and other possible fungi. The analysis by means of optical microsope of the different forms of mycorrhizae allows their identification, however this needs to be confirmed by biomolecular analysis.

This verification is important especially when there is analysis of truffle plants which produce mycorrhizae similar to other types such as *Tuber mesentericum* Vittad. and *T. aestivum* Vittad.

A2 – Evaluation of the degree of mycorrhization of a plant by means of taking into account the root tips.

Select from the root apparatus, considered as a whole, starting from the collar up to its extremities, two sections of equal length: one proximal and the other, distal.

Extract from two different points distant from each other, 6 root portions in the proximal midpoint of the root apparatus and 6 portions in the distal midpoint.

Starting from the proximal part of the collar of every root portion, you count, with no exclusion, the first 50 root tips separating them into:

- mycorrhized by declared truffle species;
- mycorrhized by other fungal species;
- mycorrhized by other truffles;
- non-mycorrhized.

Calculate, for each sector of the entire plant, the percentage of tips mycorrhized by the declared truffle species, of the tips mycorrhized by other fungal species, other truffles and those non-mycorrhized, referring to the total number of tips counted.

Extract 10 mycorrhizae morphologically ascribable to the *Tuber* genus to be placed under biomolecular examination.

COMMENT A2 – The counting of mycorrhizae in portions of roots allows for the provision of numerical data necessary in case of contestation.

This method of control, however, does not always allow the evaluation of the real percentage of tips mycorrhized by truffle: in previous work it was concluded that in order to have the correct percentage it would be necessary to count all of the root tips subdividing them into mycorrhized by truffle, by other fungi, other truffles, and bare tips.

For this reason, in respect to the method of 1995, the number of root portions to be counted has been increased.

A3 - Criteria for the definition of the suitability of a mycorrhized plant.

On the basis of many years of experience in the observation and analysis of mycorrhized plants it is held opportune to consider a plant appropriate for truffle cultivation when it maintains contemporaneously the following requisites:

a) a percentage of declared truffle mycorhrizae equal to or greater than 30;

b) a percentage of tips mycorrhized by fungi different from that of the declared truffle and not greater than 10;

c) the absence of mycorrhizae produced by other *Tuber*;

d) the difference between the percentage of tips mycorrhized by the declared truffle and that of tips mycorrhized by other fungi is equal to or greater than 30;

e) characteristics of health and development of the aerial and root apparatus correspond to CE norms for forest nursery production;

the root apparatus is not excessively spiralled (fig. 1).

COMMENT A3 – In response to the question: how many root tips mycorrrhized by truffle must a truffled plant have so that it is able to produce truffles once planted? At this stage of knowledge we can only say that the more it has, the better. To establish the minimum percentage reference was made to the 12,000 analyses carried out in nurseries.

The one year old plants (7-9 months after innoculation) are commonly poor in mycorrhizae and only 45% of these reach the minimum limit of 30% mycorrhization. In the plants of two or more years, seeing that the percentage of mycorrhization commonly increases with age, the limit could increase to 40%.

Another problem is represented by alien fungi which shouldn't be there; also in this case experience was gained in thousands of examinations. In the nursery some species such as the genus *Sphaerosporella* is often found, but it is rarely found in the field. Also considering that other fungal species common in the field were quite rarely found , there is a tollerance of a limited percentage of alien fungi.

Diseased plants are not tolerated, nor those not balanced in the aerial/ root apparatus ratio, as well as those with greatly spiralled roots or maintaining mycorrhizae produced by other *Tuber* that give evidence to poor attention to the control of the inoculation on the part of the nursery worker

B – EVALUATION OF THE DEGREE OF MYCORRHIZATION OF A HOMOGENEOUS LOT OF PLANTS DESIGNATED FOR TRUFFLE CULTIVATION

B1 – Homogeneous lot

A lot may be defined as homogeneous if it is made up of not more than 500 plants of the same species and age, obtained by equal propogation methods, innoculated with the same method over a period of time not greater than one day, using the same inoculation and cultivation(with the same technique) in the same growing environment.

In order to obtain better homogeneity of the lot, any plants with damage to the aerial apparatus damaged by parasites are eliminated in addition to those that show deformed development compared to the other plants or that cannot be considered suitable according to the nursery profile.

COMMENT B1 – The size of the lot is limited to 500 specimens primarily for two reasons:

a – in greenhouses the environment is not homogeneous and for this reason a change in mycorrhization can be verified in large lots that occupy different parts of the same greenhouse;

b – the nursery worker is favoured because if a lot is not certified the damages are limited to 500 plants.

B2 – Sampling method.

The degree of mycorrhization of the lot is evaluated on sample plants because the analysis consequently results in damage to the plant examined..

In homogeneous lots the sampling must be carried out by collecting:

1% (overcalculated) of the plants present in the lot plus 5.

The individual plants that make up the analysis sample are collected at random by the analyst or along pre-established lines.

The sampled plants, while waiting to be analysed, should be transferred to the laboratory of the organization responsible for the examinations and should be given normal cultivational care to preserve complete integrity.

Every plant of the sample must be analysed following the fore-mentioned methodology. The values found for each plant are recorded on a chart referring to the lot analysed(chart a)

The ten mycorrhizae extracted from each plant are gathered in a single sample which is placed under biomolecular analysis.

B3 - Criteria for the definition of the suitability of a plant lot.

On the basis of experience in the examination of numerous homogeneous plant lots taken from different greenhouses and in the consideration of biological order, it is agreed that a homogeneous lot may be declared suitable for the purpose of truffle cultivation when all of the plants of the sample are qualified in accordance with the requisites presented at point A3.

A tollerance of one plant that does not conform to the parameters presented at point A3 is allowed, as long as there is the presence of the declared truffle and it is without mycorrhizae produced by other truffles.

If on analysis the lot does not result suitable, the plants that make it up can be placed under further examination.

C – DURATION OF VALIDITY OF ANALYSIS CERTIFICATES

During the time spent by a plant in a greenhouse, the state of mycorrhization can change in a positive or negative sense for which the certificate of mycorrhization has a maximum validity of one year even if the plant is maintained in the same environment .

Should this be the case, certified plants grown even for short periods in different environments must be placed under further examination.

COMMENT C – The one year validity of the certificate of mycorrhization is due to the fact that the plants over the said period can modify their mycorrhization in a positive or negative sense.

D – THE MAXIMUM AGE OF A MYCORRHIZED PLANT

A plant kept in a container results in the increase of root mass over time, and the consequent malformation of some roots which could cause a minor abilility to take root growth and resistance in the field.

The analyst needs to evaluate the analysed plant's capability of development dicarding those plants with highly spiralled root apparatus. .

COMMENT D – Truffle-infected plants need to be young and healthy in order to properly take root following transplant and to yield a plant that is sound, hardy, and resistant to environmental adversity. Plants grown in small containers can develop a deformed root apparatus which persists even after the transplant.

The task of the analyst is to discard plants, even if well-mycorrhized, that do not guarantee the formation of a healthy tree.

In conclusion, it is hoped that the constitution of a commission formed by researchers from all of the European truffle producing nations, who taking this proposal as a basis, could formulate a method to adopt in all of Europe.

THANK YOU FOR YOUR ATTENTION

Tab. n° ……

Nursey …….…… Greenhouse no. …… Lot no. …… No. Plants present…….

Symbiont plant ……..…… Truffle inoculated ……..……..

Pianta	% micorrize				validity forest		Sample no.
n°	*Tuber* inoculated	other *Tube*r	other fungal	no micorrìhized	si	no	An. Biomol.
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Giudizio: