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Short note

Determination of the peridium thickness of *Tuber magnatum* ascomata from Molise region

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Abstract
Several studies focused on *Tuber magnatum*, the most valuable truffle species, with a limited geographical distribution. However, no comprehensive information on the thickness of its peridium (the external surface of fruiting bodies) is available. Accordingly, to fill this lack of information and in order to provide a more in-depth morphological characterisation of white truffle populations from Molise region (one of the Italian richest areas of truffles), the peridium thickness of twenty-one *T. magnatum* ascomata collected from two different study sites was measured by light microscope observations. A considerable variability within the analysed populations emerged, with values ranging from 271.25 µm (minimum) to 1231.25 µm (maximum), and an average peridium thickness of 622.33 µm. Interestingly, significant differences were observed between the two groups, with truffles harvested in an inner area of the region showing a peridium significantly thinner than those collected on the border with Abruzzo region. No linear correlation between peridium thickness and other morphological parameters (ascoma weight and maturity) emerged. It is likely that the differences observed between the two *T. magnatum* populations could be related to factors such as soil characteristics, site-specific features, genetic traits, as well as truffle collection period, which should be properly investigated.

Keywords
white truffle; fruiting body; external surface; morphological traits; light microscope; Central-Southern Italy

Introduction
Tuber magnatum Picco is an ectomycorrhizal ascomycete of the *Pezizales* order that, in symbiosis with several trees and shrubs, produces edible hypogeous fruiting bodies, the so-called Italian white truffles, one of the most expensive foods in the world (thousands of euros per kilogram; Laruccia et al., 2020). In addition to the valuable culinary properties, the exorbitant prices of *T. magnatum* ascomata reflect their low availability on the market, which is related to both the difficulties in cultivation and the limited distribution range (Mello et al., 2006; Christopolous et al., 2013; Iotti et al., 2014; Riccioni et al., 2016). Indeed, this prized truffle species, for a long time considered endemic to Italy, grows spontaneously only in a few European countries (Rubini et al., 2005; Belfiori et al., 2020).

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Molise region (Central-Southern Italy) is one of the most productive area of the Italian peninsula. Indeed, it is estimated that the total amount of *T. magnatum* truffles annually collected in the Molise forests is between 30 and 70 quintals (https://www.agi.it/lifestyle/news/2021-01-11/molise-diventa-patria-tartufo-10978317/). However, although the prized white truffle has been extensively characterised from many points of view, Molise truffles have received very little scientific attention (Monaco et al., 2020, 2021).

Several studies focused on *T. magnatum* phylogeography and genetic characteristics (Mello et al., 2005; Rubini et al., 2005; Belfiori et al., 2020; Monaco et al., 2021), associated microbial communities (Barbieri et al., 2007, 2010; Amicucci et al., 2018; Monaco et al., 2021; Niimi et al., 2021), volatile organic compounds (Vita et al., 2015; Niimi et al., 2021), molecular and biochemical traits (Vita et al., 2020), ecological features (Marjanović et al., 2015), and cultivation techniques (Bach et al., 2021).

Nevertheless, to date, very few data relating to the thickness of the peridium (the external surface of truffle fruiting bodies) are available.

The peridium takes on different features depending on *Tuber* species. In the black truffles *Tuber aestivum* Vittad. and *Tuber melanosporum* Vittad., it is brown-black and characterised by the presence of hard pyramidal warts; in *Tuber borchii* Vittad. it appears at first whitish, then greyish-yellow and finally brown ochre in colour, quite pubescent when immature and, at maturity, glabrous, humid, and smooth, often with darker or lighter reddish spots, whereas *T. magnatum* peridium is smooth, suede-like, and adherent to the gleba (the inner part of the ascoma) (Hall et al., 2007; Angelini et al., 2016).

This short note reports the preliminary results of peridium thickness measurements of two white truffle populations (*T. magnatum*) harvested within natural truffle grounds in Molise region, with the aim to 1) fill the lack of information on this parameter and 2) provide a more in-depth morphological characterisation of the Molise white truffles.

Materials and Methods

Sampling

In this study, twenty-one ascomata belonging to two distinct *T. magnatum* populations were considered. They were collected in two different areas of Molise region (Fig. 1). In particular, nine ascomata (samples from 1 to 9, group 1) were harvested in November 2019 in the study site 1, between Carovilli and Vastogirardi municipalities (Isernia province), an area highly suited to truffle collection (Paolanti et al., 2014; https://www.agi.it/lifestyle/news/2021-01-11/molise-diventa-patria-tartufo-10978317/). Other twelve samples (indicated with progressive numbers from 10 to 21; group 2) were collected in January 2020 in the study site 2, a more extensive area on the border with Abruzzo region. Truffles were found, with the help of trained dogs and expert truffle hunters, in natural orchards located within mixed coppice woods, with a vegetation composed mainly of hazel trees (*Corylus avellana* L.), Turkey oaks (*Quercus cerris* L.), beeches (*Fagus sylvatica* L.), and cornels (*Cornus sanguinea* L.). Then, they were placed in polypropylene containers and transported to the laboratory under refrigerated conditions (Monaco et al., 2021).

Dried samples of each specimen were deposited in the herbarium at the University of Molise, Italy.

Determination of peridium thickness

Tuber magnatum ascomata were gently brushed and washed with distilled water to remove soil residues. In order to determine the thickness of the peridium, fruiting bodies were divided in half and cut lengthwise with a steel blade to obtain thin sections. For each truffle, five sections were considered by taking the peridium in several points of the ascoma. In addition to the peridium, sections also included a small portion of gleba to facilitate the subsequent determination of the peridium thickness by microscopic observation (Fig. 2). The slices so obtained were placed on a microscope slide and covered/wetted with distilled water before applying a coverslip. The thickness of the peridium was

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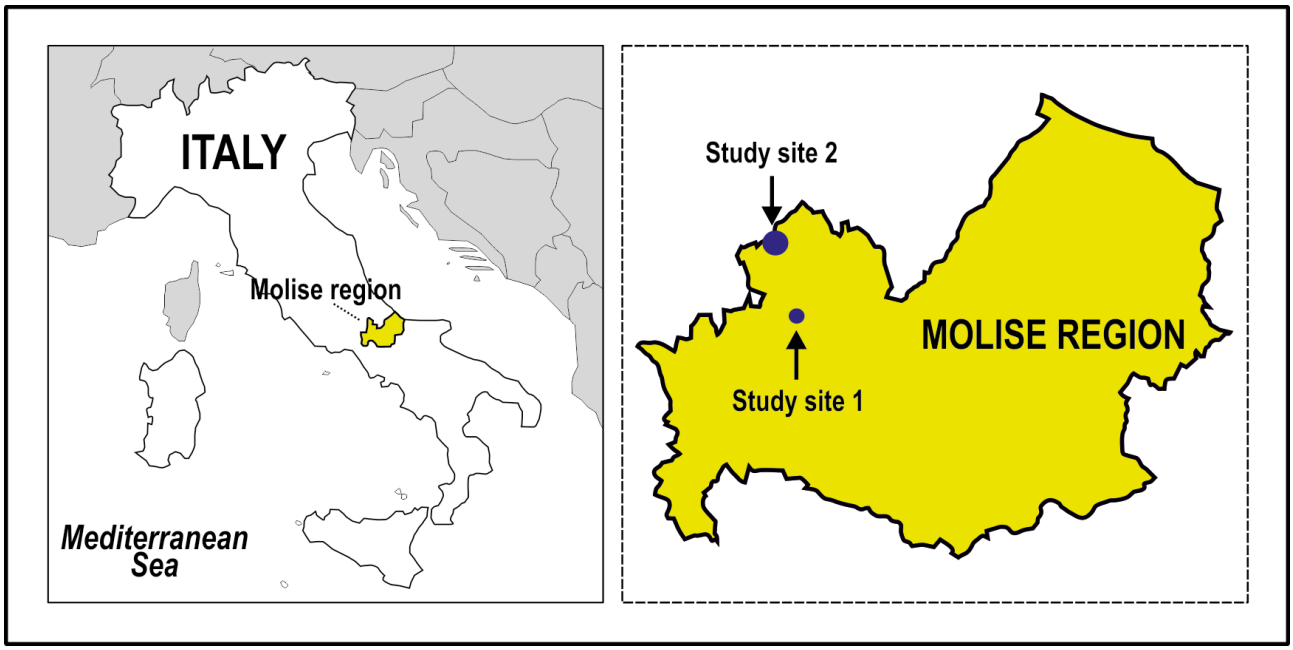

Fig. 1 - Study area. The two collection sites are shown on the map: the study site 1 is located between Carovilli and Vastogirardi municipalities whereas the study site 2 covers a more extensive area on the border with Abruzzo region.

determined with the aid of a light microscope equipped with a ruler (Leica Microsystems, 10× objective). For each fruiting body, it was measured in twenty-five different points (5 for each section), positioning the microscope ruler perpendicularly to the section to be analysed (Fig. 2). The average thickness of the peridium was then determined by calculating the arithmetic mean of the twenty-five measured values.

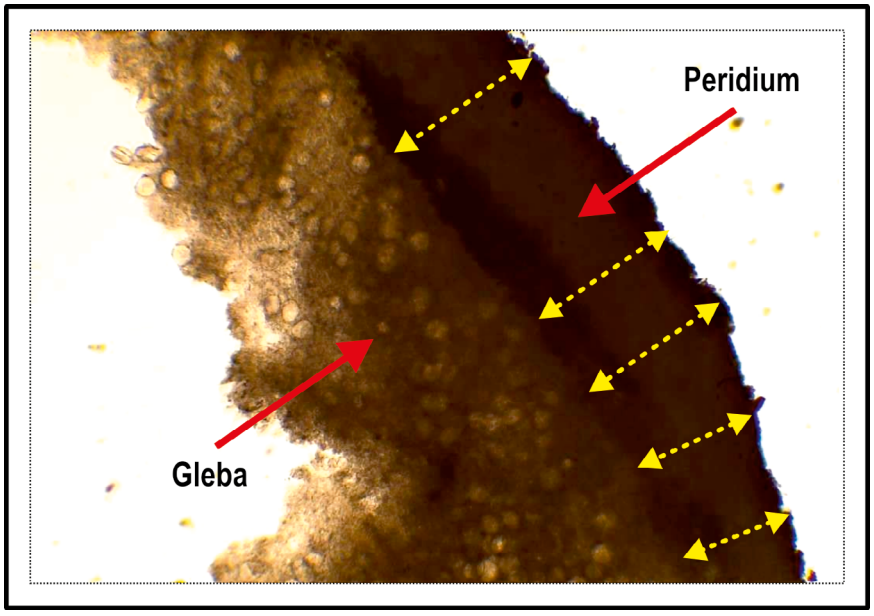

Fig. 2 - Example of peridium thickness determination by microscope observation of a thin truffle section also comprising a small portion of gleba. As shown by the yellow arrows, for each section the thickness of the peridium was measured in five points to obtain a total of 25 values for each ascoma.

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In order to assess whether there were statistically significant differences in the peridium thickness of the two investigated *T. magnatum* populations, Student's t-test was performed. Moreover, an analysis with the Pearson's correlation coefficient was conducted in order to verify the possible linear correlation between peridium thickness and ascoma weight and maturity [these two morphological data are reported in Monaco et al. (2021) for the same samples here analysed].

Results and discussion

Even if several works exist on the prized white truffle, to date, no comprehensive information on the thickness of fruiting body peridium is available. Indeed, there is a lack of systematic studies aimed at analysing this aspect. Some of the few data found in scientific literature have been reported by Zambonelli et al. (2000), who examined this characteristic for 5 samples of *T. magnatum* from Emilia Romagna region, and by Angelini and colleagues (2016), as part of a more extensive work mainly focused on the isolation and identification of allelochemicals from *Tuber* ascomata. Consequently, these Authors, having different goals, did not investigate the peridium thickness in relation to other parameters (such as, for example, fruiting body size or maturity, truffle collection period, etc.).

Accordingly, to fill this lack of information and in order to provide a more in-depth morphological characterisation of the Molise white truffles, the peridium thickness of the twenty-one ascomata collected from two different areas of the region, between November 2019 and January 2020, was accurately measured by light microscope observations.

The two examined *T. magnatum* populations were previously included in a wider research, in which they were analysed from a morphological, genetic, and microbiological point of view, highlighting a surprising heterogeneity between and within the investigated groups (Monaco et al., 2021). Although, in agreement with prior investigations (Büntgen et al., 2017; Garcia-Barreda et al., 2021), no strong linear correlation between fruiting body weight and maturity was found, truffles of the first group were, overall, of greater dimensions and riper compared to those of the second group (Monaco et al., 2021).

Measurements of the fruiting body peridium carried out in this study further confirmed the heterogeneity detected since, as shown in Table 1, a considerable variability within the analysed populations emerged, with values ranging between 271.25 µm (minimum) and 1231.25 µm (maximum).

Overall, the peridium thickness of the examined Molise truffles was on average 622.33 µm. This mean value differs from those recorded by Zambonelli et al. (2000), for samples collected in Northern Italy, and by Angelini et al. (2016).

Interestingly, significant differences were observed between the two investigated populations (Student's t-test, $p < 0.01$): indeed, truffles harvested between Carovilli and Vastogirardi municipalities (group 1) showed a peridium significantly thinner than those of the group 2, with average values of 452.42 and 749.76 µm, respectively.

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Table 1 - Average peridium thickness of the twenty-one white truffles (*T. magnatum*) from Molise region.

SAMPLE CODE	GROUP	AVERAGE PERIDIUM THICKNESS* (µm)
1	1	298.75
2	1	271.25
3	1	287.68
4	1	521.36
5	1	487.00
6	1	667.50
7	1	482.22
8	1	533.50
9	1	522.50
10	2	1231.25
11	2	813.75
12	2	985.16
13	2	444.29
14	2	798.75
15	2	653.13
16	2	757.50
17	2	394.17
18	2	637.67
19	2	854.46
20	2	615.91
21	2	811.03

*The average peridium thickness of each fruiting body was given by the arithmetic mean of the twenty-five measured values

The analysis with Pearson's correlation coefficient revealed that there is no linear correlation between truffle weight and peridium thickness (r = - 0.21) and only a slight/moderate negative relationship between fruiting body maturity and the thickness of the peridium (r = - 0.50).

In our opinion, it is likely that the differences observed in the peridium thickness between the two analysed *T. magnatum* populations, similarly to the differences previously reported for truffle weight and maturity (Monaco et al., 2021), could be related to the diverse collection period of the ascomata (fruiting bodies of the first group were collected in November whereas those of the second group in January). Indeed, it is known that the white truffles of January can be morphologically different from *T. magnatum* collected between October and December, because of the ripening in winter period, under frost and ice (http://www.artopoltrepo.it/Documenti/manualetartufi.pdf). Therefore, we might assume that the thicker peridium of the fruiting bodies harvested in January 2020 (group 2) could represent a kind of adaptation to unfavourable environmental conditions. However, since no comparison data are available, further analyses will be required to shed light on an effective correlation between peridium thickness and environmental/climatic conditions. On the other hand, the remarkable variability detected within the examined *T. magnatum* populations could be explained taking into account other factors that should be properly investigated, such as genetic traits, site-specific features, soil characteristics, and microclimatic conditions.

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Conclusion

In the future, it would be interesting to carry out further analyses including a larger number of ascomata collected in different areas of the region and in other Italian regions, also to verify the existence of a possible link between the peridium thickness and *T. magnatum* provenance. Indeed, the potential use of the peridium thickness as an effective parameter to track the geographical origin of truffles could have a significant impact on the economy related to this product (mainly at regional scale) from different perspectives (truffle traceability, promotion and conservation), and represents a relevant aspect to be properly investigated in future researches.

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