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MORPHOLOGICAL CHARACTERISTICS OF SUMMER TRUFFLE (*TUBER AESTIVUM* VITTAD.) FROM BOSNIA AND HERZEGOVINA

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Abstract

The aim of this paper is to present as accurately as possible the morphological characteristics of the summer truffle, found in Bosnia and Herzegovina, and to compare them with the characteristics of the previously described specimens in other countries and regions.

Well-developed fruiting bodies of summer truffles (*Tuber aestivum* Vittad.) were found in oak and beech forests on calcareous soils in Bosnia and Herzegovina with the help of trained dogs.

On the basis of a representative sample, composed of specimens, collected during three consecutive truffle seasons, the following were analyzed: shape, size and mass of ascocarps, structure of the harvest, gleba color, size and shape of ascospores.

It was found, that predominant geometric shape of their fruiting bodies is ellipsoid with an average mass of 44.3 g. Most acocarps weighed less than 20 g. As the weight of the pieces increases, their frequency decreases, so that ascocarps over 60 g participate in number with only 20 % and by weight with 47.2 % of the total yield. The length of spores ranges from 26.0 to 35.8 μm, with an average of 30.3 μm, while the width of spores varies from 17.2 to 26.1 μm, with an average of 21.3 μm. The Q-shape parameter takes values in a wide range from 1.16, which roughly corresponds to the globular shape, to 1.64 for the distinctly ellipsoidal shape.

In sum, the shape and size of the summer truffle ascocarps and spores native to Bosnia and Herzegovina fit the general picture of this taxon.

Keywords: summer truffle, size and mass of ascocarps, crop structure, size and shape of ascospores.

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1. Introduction

Due to the fact that very heterogeneous phytogeographical, ecological, geomorphological and hydrological conditions are encountered in a geographically small area, Bosnia and Herzegovina (B&H) is characterized by a high species diversity. However, the achieved level of biodiversity exploration is very low, especially in the kingdom Fungi. It is assumed, that about 15,000–20,000 species of fungi live in B&H, and until 2009 only 552 species were described [1], among which there was no representative of the genus *Tuber*. In the meantime, a monograph on mushrooms from the order *Pezizales* was published, describing only one species of truffle, *Tuber excavatum* Vittad [2]. Apart from this species, only one other representative of the family *Tuberaceae* has been registered in B&H. It is hog truffle (*Choiromyces meandriformis* Vitt.) [3]. When it comes to foreign sources, samples of *Tuber aestivum* were analyzed in two recent papers, dealing with elemental composition and truflle aromas [4, 5].

In the last few year researchers from two universities in the city of Banja Luka, assisted by members of the NGO SENSE, found four species of truffles in B&H, including the species *Tuber aestivum.* Under this name, the species was first described in 1831, in the work [6]. Before, it was the author of [7] in 1823 who described this truffle as *T. albidum*, with an emphasis on *verrucis exasperatum, albidum*. The German Mycological Society (DGfM – Deutsche Gesellschaft für Mykologi Society) lists the following synonyms: *Aschion nigrum* Wallr. 1833, *Tuber blotii* Deslandes 1824, *Tuber bohemicum* Corda 1854, *Tuber culinare* Zobel 1854, *Tuber gallicum* Corda 1854, *Tuber uncinatum* Chatin 1888. In addition to the above, The Global Biodiversity Information Facility (GBIF) also mentions these: *Lycoperdon aestivum* Wulfen 1787, *Rhizopo aestivus* Fr., *Hymenangium aestivum* (Wulfen) Rabenh. 1844, *Tuber aestivum* var. *aestivum* (Wulfen) Spreng. 1827, *Tuber aestivum* var. *gallicum* (Corda) Sacc., *Tuber aestivum* var. *meridional* G. Chev. & Riousset, *Tuber aestivum* var. *uncinatum* (Chatin) I. R. Hall, P. K. Buchanan, Y. Wang & Cole. If the International Code of Botanical Nomenclature (ICBN) was strictly applied, the name *T. albidum* Fries, since the oldest name, should take precedence over the name *T. aestivum*. However, because *T. albidum* has not been used for many years, its reincarnation is practically no longer possible [8]. *T. aestivum* is also known under common names as summer truffle, black summer truffle and Burgundy truffle; the red-grained black truffle is less common name. Gastronomers, traders and many mycologists emphasize the difference between summer and Burgundy truffles, with Burgundy truffle being considered as a late season taxon of higher quality and value. The unknowns and doubts around the two taxa (morphotypes/varieties/species) and the corresponding scientific nomenclature go beyond the scope of this paper.

Summer truffle is considered the most common truffle in moderate climate of middle European countries and the most valuable species after white truffle (*T. magnatum* Picco) and Périgord black truffle (*T. melanosporum* Vittad.) [9, 10]. As such, summer truffle is also a species of potential economic importance for the rural population, on condition that its exploitation is carried out in a responsible and sustainable manner.

The aim of this paper is to provide informations on morphological characteristics of summer truffle, originating from B&H, such as: shape, size and mass of ascocarps together with structure of the harvest, gleba color, morphological and biometric characteristics of spores. Knowing the shape and mass of a piece is not an end in itself. It is an argumentative stronghold for the possible prescribing of the minimum allowed dimensions for collection, analogous to the minimum hunting dimensions in fishing, for example. If such approach had been implemented in practice earlier and more broadly, we would not be lamenting today how we had expressed yield in tons a hundred years ago and today we express it in kilograms [11–13].

2. Materials and Methods

2. 1. Finding truffles and sample formation

Fruiting bodies of the summer truffle were found in oak and beech forests on calcareous soils, with the help of trained dogs of the Laggoto Romagnolo breed. Most of these forests are managed by the Forest estate "Gorica" Šipovo, while a smaller part is privately owned. Fruit bodies of *T. aestivum* were collected over three consecutive fruiting seasons between August 2018 and November 2020 (exact collection dates are given in **Table 1**).

The sample consisted of 30 fruit bodies, selected in a systematic manner, – one in five in the order of finding.

The original monograph from 1831 was used to identify the taxon [6]. The additional taxonomic verification of the collected material was performed using works [14, 15].

Table 1
Mass and volume of *Tuber aestivum* ascocarps collected in three consecutive season

Piece No.	Sampling date	Mass (g)	Dimension (mm)			Volume (cm^3)	Specific volume (g/cm^3)
			a	*b*	*c*		
1	23.08.2018	30.9	44	41	36	34.0	0.91
2	23.08.2018	43.5	48	47	40	47.3	0.92
3	23.08.2018	95.1	74	53	52	106.8	0.89
4	17.10.2018	78.4	60	55	45	77.8	1.00
5	17.10.2018	54.6	55	45	38	49.3	1.11
6	17.10.2018	11.9	34	27	24	11.5	1.03
7	17.10.2018	10.9	30	27	27	11.5	0.95
8	20.10.2018	55.7	60	50	40	62.9	0.89
9	20.10.2018	29.6	50	38	36	35.8	0.83
10	20.10.2018	23.3	50	33	30	25.9	0.90
11	20.10.2018	22.1	40	40	30	25.1	0.88
12	20.10.2018	19.1	34	34	30	18.2	1.05
13	20.10.2018	14.7	36	32	26	15.7	0.94
14	20.10.2018	14.8	30	30	30	14.1	1.05
15	07.10.2019	40.1	50	44	40	46.1	0.87
16	22.10.2019	13.3	35	28	25	12.8	1.04
17	22.10.2019	23.2	49	32	31	25.5	0.91
18	22.10.2019	55.2	56	46	39	52.6	1.05
19	22.10.2019	12.3	31	29	28	13.2	0.93
20	22.10.2019	25.2	38	37	36	26.5	0.95
21	10.10.2020	45.3	48	46	42	50.9	0.89
22	10.10.2020	40.5	56	38	36	40.1	1.01
23	10.10.2020	82.4	62	54	50	87.7	0.94
24	10.10.2020	24.7	49	32	31	25.5	0.97
25	10.10.2020	70.7	60	52	42	68.6	1.03
26	02.11.2020	45.3	51	43	41	47.1	0.96
27	02.11.2020	218.9	87	73	70	232.9	0.94
28	02.11.2020	82.3	60	52	48	78.4	1.05
29	02.11.2020	26.2	39	35	33	23.6	1.11
30	02.11.2020	19.2	42	31	29	19.8	0.97
	Σ	1329.4	–	–	–	1387	29.0
	St	7.45	–	–	–	7.89	0.0134

2. 2. Determining shape, size and mass of ascocarps

When observing ascocarps, it should be taken into account, that the soil, in which they are formed, is a compact medium. There are small pores in it, but rare fractures or pockets are reserved to accommodate acocarps. Truffles need to make a significant effort "to find their place underground", unlike aboveground carpophores, which do not have such challenges. Therefore, in truffles, as distinct from fruit, for example, we cannot talk about pear-shape, apple-shape or some other recognizable more or less unified shape, but rather a range of shapes from spherical, elliptical to kidney-shaped, heart-shaped and various complex shapes.

The maximum length or longitudinal dimension was measured for each piece (marked as *a* in **Fig. 1**). Then the dimension perpendicular to the previous one (*b*) was measured. Finally, the maximum width or transverse dimension (*c*) was taken; all dimensions are expressed in millimeters.

Depending on their relative relations, the volume of the piece is approximated with sphere (when $a \approx b \approx c$), with prolate ellipsoid (when $a>b \approx c$) and with ellipsoid (when $a>b>c$). The volume was calculated using standard equations:

$$V_1 = 4/3 \cdot \pi \cdot a^3, \quad (1)$$

$$V_2=4/3\cdot\pi\cdot a\cdot b^2, \quad (2)$$

$$V_3=4/3\cdot\pi\cdot a\cdot b\cdot c. \quad (3)$$

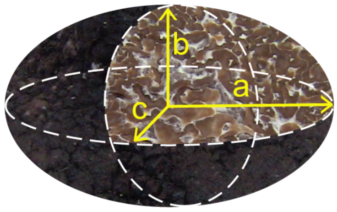

Fig. 1. Approximation of the volume of ascocarp

2. 3. Analyzing structure of the harvest

Individual mass of ascocarp is a variable characteristic that can be approximated by an average value. This data first of all tells us how many pieces there are in one kilogram of yield, then indicates the expected maturity of the collected pieces and speaks about the compliance of yield with the needs or desires of the end user – chefs typically request 30 to 50 g "golf balls" in regular round shape [16]. A more complete insight into the yield structure was obtained by arranging the sample elements according to their mass and then summarizing them into classes 10 g wide.

In a situation when we still do not have own database, nor valid data on the size and structure of yields in natural stands from other (foreign) sources, we compared our harvest structure with the structure, achieved in artificial summer truffle orchards [17]. It is about plantations of common oak (*Quercus robur*) and Turkey oak (*Q. cerris*) in a mixture of 70:30 % and a planting density of 4,000 trees/ha (the distance between rows is 2.5 m and 1 m in a row). From this data alone, it can be concluded, that this base is not perfect because the number and arrangement of trees is inappropriate to modern principles of summer truffle plantations. According to the author of [18], the minimum planting distance for *Q. robur* is 4×4 m, which means a maximum of 625 trees/ha, while according to the author of [19], the intended distance is 4×5 m or a maximum of 500 trees/ha. For *Q. cerris*, a slightly higher planting density is recommended: 4x3 m or a maximum of 833 trees/ha [18]. In this case, it is almost certainly a matter of subsequent mycorrhization of the previously established short-rotation coppice (energy plantation), whose primary goal is the production of raw material for solid biofuels (hog fuel, wood chips, etc.) but not truffles [20]. Otherwise, the yield structure is directly reflected in the rationality of production, sustainability of yield and maintenance of species.

2. 4. Gleba color

Color is one of the essential characteristics of all living world. While Mother Nature was immensely generous to the above-ground subjects of the kingdom Fungi (just to mention representatives of genera *Amanita*, *Boletus* and *Russula*), towards underground inhabitants it was much more stingy, which does not diminish the importance of knowing their color. In underground fungi with reduced coloring, recognition and accurate citation of their color is even more important and challenging.

For the purposes of a research, the color of the observed object is determined mainly in three ways. The first method is performed using instruments and the procedure itself is known as colorimetry. The second method is performed by comparing the color of the observed object with the color scale in one of the recognized catalogs, until we come across an identical or approximately similar color. This procedure requires a lot of time and patience and is fraught with the error of subjective assessment. The third procedure is faster, simpler and less subjective. It is performed using software that scans the color of the captured object and compares it with the established color database. We determined the cross-sectional colors of the summer truffle in this (third) way. We used a Hexadecimal base/color scale (HEX) [21] with a corresponding description [22]. A millimeter raster grid was laid over 5x enlarged image and then the relative representation of four colors with the highest frequency was calculated, two for conditionally speaking dark fields, where reproductive organs (spores) were formed and two for conditionally speaking light fields, where gleba remained sterile. In this way, in addition to the color itself, basic knowledge was gained about the reproductive potential of summer truffles, and the relationships between color, maturity and age of ascocarps.

2. 5. Size and shape of ascospores

Sections, mounted in 5 % KOH and lactophenol cotton blue, were made from fresh ascomata. 25 asci with four spores were selected in random manner, a total of 100 spores. Asci that contain a different number of spores (less or more than four) were not taken into account because most authors observe only four-spores asci in such measurements. From **Fig. 2** we can see that spores from the sac with a smaller number of observed elements are, as a rule, larger, and from the more numerous, smaller. Therefore, opting for an equivalent number of spores in all observed asci, achieves a better and more objective comparison.

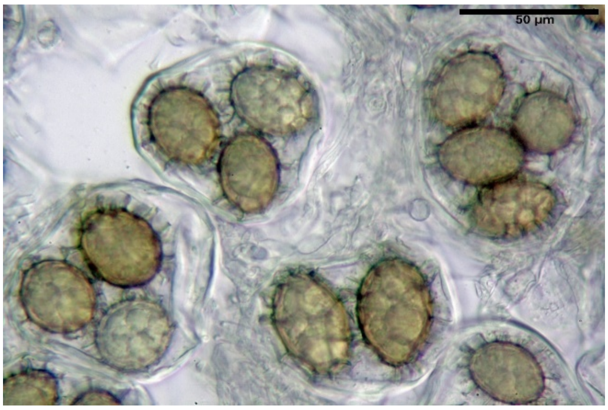

Fig. 2. Ascii with ascospores of truffle native to B&H, bar=50 µm

Only mature and fully developed spores were measured. Underdeveloped or damaged spores were not taken into account. The ornamentation of the spores was not taken into account, as well. Spores from fresh ascocarp were examined microscopically. The observation was performed by mean of a light microscope, equipped with a drawing tube and a scaled ocular. Line drawings and measurements of the ascospores were performed at 1000x magnification under normal light. Two dimensions were measured on the sample of 100 spores: length (l) along the spore and width (w) across the spore. The quotient $Q=l/w$ is a calculated variable (it did not arise as a result of some measurement) that describes the shape of the ascospore. A high value of Q means narrow spores, while a low value of Q means broad spores. The spore mass color was observed using a digital microscope at 80x magnification.

All statistical data processing was done using free statistical software "jamovi 1.6.3.0".

3. Results

Fruiting bodies of observed truffles are dark brown to black, ornamented with pyramidal warts, usually intersected with angular fissures and sometimes with less pronounced transverse wrinkles. Their scent is mild, similar to mushrooms, closest to the smell of porcini mushrooms. They taste like nuts with a distinctive note of chives.

3. 1. Shape, size and mass of ascocarps

The average weight of ascocarpis is 44.3 g and varied in the range of 10.9–218.9 g (**Table 1**). This means that in one kilogram of fresh truffles from B&H there are about 23 pieces. The predominant geometric shape of fruiting bodies is ellipsoid (ascoma No. 9, 10 and 12, **Fig. 3**), followed by spherical (geoid) forms (ascoma No. 13 and 14), while the least represented are complex geometric bodies, such as heart-shaped (ascoma No. 8 and 11) kidney-shaped and other forms.

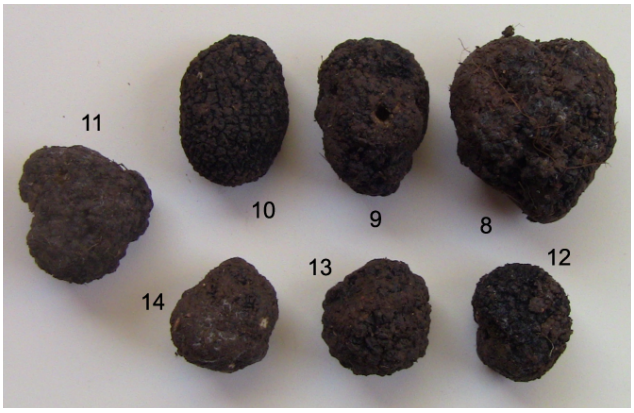

Fig. 3. Summer truffles harvested on October 20, 2018 (ascoma`s labels correspond to the numbers in **Table 1**)

Most ascoma (26.7 %) has a mass of less than 20 g, i. e., between 10–19 g, since pieces less than 2 cm in diameter were not collected. At the same time, this mass class gives only 8.7 % of the observed yield (harvest), (**Table 2**). As the individual mass increases, the numerical frequency decreases. After the breakpoint of 50 g, there are fewer and fewer larger pieces, but at the same time their share in the total yield is increasing, so that ascoma above 60 g participate in number with only 20 % and by mass with 47.2 % of the total yield. If we look at this rule of piece mass from a broader, production-technological aspect, we can conclude that harvesting ascocarps lighter than 10 g, or less than 2.5 cm in diameter is not rational due to high time consumption per unit of production in all phases of work, from harvest to the kitchen or factory, nor is it justified from the aspect of sustainability of yield and survival of the species.

Table 2
Distribution of ascocarps by mass classes

Absolute and relative frequency (%)	Mass classes (g)						Total
	10–19	20–29	30–39	40–49	50–59	60>	
Total number of pieces	8	7	1	5	3	6	30
Total mass (g)	116.2	174.3	30.9	214.7	165.5	627.8	1329.4
Relative abundance of ascocarps (%)	26.7	23.3	3.3	16.7	10.0	20.0	100
Relative mass of ascocarps (%)	8.7	13.1	2.3	16.2	12.5	47.2	100

A strong linear correlation was established between the calculated volume and the measured mass of ascocarp, which can be approximated by the equation:

$$m=0.810+0.941 \cdot V, \quad (4)$$

where m – mass (g); V – volume (cm^3); r=0.997 and r^2=0.993, with p<0.001.

The specific weight of ascocarp was 0.966 g/cm^3, and it ranged from 0.83 to 1.11 g/cm^3.

In the absence of adequate parameters from Bosnia and Herzegovina, we will compare the structure of the studied yield with the structure of the yield achieved in the Jászivány orchard in central Hungary [17]. In 2014 and 2015 seasons, a total of 7451 ascocarps were collected and a yield of 178.8 kg was achieved, with an average weight of 24 g per piece. According to mass classes, the total number of collected fruiting bodies was distributed as follows:

– in the class 0–9 g there were 2278 pieces or 30.6 %;
– in the class 10–19 g: 2060 pcs or 27.6 %;
– in the class 20–29 g: 1265 pcs or 17 %;
– in the class 30–89 g: 1647 pcs or 22.1 %;
– in the class 90 g>: 201 pcs or 2.7 %.

Compared to the studied distribution, the Hungarian distribution has shifted to the left, mostly because even the smallest pieces, weighing less than 10 g, were collected, so that over 4,200 pieces (58.2 %) had a mass of less than 20 g. As a result, the average weight of pieces in Hungarian truffle orchards is almost half that of the average weight of pieces in Bosnia's natural habitats.

3. 2. Gleba color

The gleba color palette in three different seasons and months of harvest is shown in **Fig. 4.** White veins as a sterile portion of gleba and brownish fertile veins, bearing spore accumulations in ascii, are visible to anaided eye. As a rule, the white veins will remain bright in color during the whole process of maturation, while the brownish portions of gleba will be darker as the spores mature, that is, as the process of their melanization progresses.

a *b* *c*

Fig. 4. Cross sections of ascocarps in three different seasons and months of harvest: *a* – 23.08.2018; *b* – 21.10.2019; *c* – 07.11.2020

In terms of maturity, the obtained results are in agreement with the views of the author of [17] that ascocarps do not mature at the same time, and that mature ascocarps can be found in a wider time-scale. However, the obtained results do not agree with the view of the same source that *weight and maturity of T. aestivum fruit bodies is mainly unrelated.* The obtained data from the field affirm the view that ascocarps with a larger mass are generally more mature, which is reflected in the dominance of dark (fertile) areas over light (sterile) zones in the cross-section of fruiting bodies. Also, if we look at **Table 3**, we will notice that in the period August-October brownish parts of the gleba cover about 75 % of the cross-section, while in November they are present on about 85 % of the surface.

Table 3

Dominant colors of summer truffle gleba with respect to harvest time

Ascocarp No.	Date	HEX color code	Color sample	Color description	Average participation (%)
1	13.09.2018	593A38		Very dark desaturated red	45
		7E6958		Mostly desaturated dark orange	30
		C8C6CB		Greyish violet	16
		E7EBDD		Light grayish green	9
2	21.10.2019	2C1D1A		Very dark (mostly black) red	60
		6A5B3C		Very dark desaturated orange	15
		A4978E		Dark grayish orange	12.5
		EBF2EB		Light grayish lime green	12.5
3	07.11.2020	3A2013		Very dark orange (brown tone)	68
		795B3F		Dark moderate orange	17
		9C9C94		Dark grayish yellow	6
		C8CBC4		Grayish green	9

At this point, it is necessary to emphasize the distinction between the terms ripeness stage and maturation stage of truffle ascocarp. Summer truffle ascocarp, like the so-called climacteric fruits, can be harvested at different maturity stages, but usually only after the first fertile veins appear, until the moment of reaching the fully ripened stage. Fully ripened ascoarps are thought to have a richer range of smells and tastes, making them more valuable. But their shelf life is also shorter, so they are more demanding for manipulation and storage. Also, truffles, taken out at the right time, must not be as hard as a stone, as this is a sign that they are not yet ripe. But as well, they should not lie down under the pressure of fingers as this is a sign that they overripe [23].

3. 3. Size and shape of ascospores

The spore mass is creamy in incompletely ripe ascoma and yellowish in ripe ones (**Fig. 5**). Asci are sessile or with short stalk, containing 1–6 spores, usually 2–4 (**Fig. 2**).

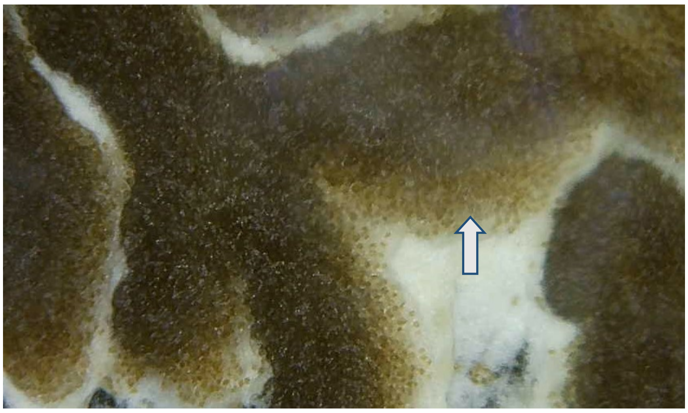

Fig. 5. Microscopic preparation (slide) of summer truffle flesh, without staining (80x); arrow shows clusters of ascii with spores

The length of the spores (l) ranges from 26.0 to 35.8 µm, with an average of 30.3 µm and a standard deviation of ±2.06 µm. The spore width (w) ranges from 17.2 to 26.1 µm, with an average of 21.3 µm and a standard deviation of +/– 1.51 µm (**Table 4**). No correlation was found between length and width. If the correlation between l and w is approximated by a linear relationship, $r^2=0.103$, which indicates a very weak explanation of the relationship. This is also understandable, given that Q-shape takes values in a wide range from 1.16 (for approximately globular forms) to 1.64 (for highly ellipsoidal forms), **Table 4**.

The average length of summer truffle spores, originating from B&H, does not deviate significantly from the length of spores from other climates (**Table 5**). A similar relation applies to the average width of the spores, except that in this case the studied sample is shifted towards the lower limit of the variation span. In terms of shape, the studied spores are less ellipsoidal compared to the third sample from Italy, which has the highest Q parameter, and they are most similar in shape to the spores from the first sample from Turkey. Overall, the shape and size of the summer truffle spores, originating from B&H, fit the dimensions of this taxon. It should be noted, that a relatively small number of papers deal with this topic, especially since spores are considered the primary characteristic of truffles [24–26].

The obtained results show that B&H has a good raw material base, at least when it comes to summer truffles, which could be used as a quality starting point for establishing managed orchards. In support of this statement, it should be added, that during the field research we did not find any sick or damaged specimens of fruit bodies.

The presented results are based on a small sample with 30 ascoscarps. Some deeper and more rigorous research should be based on a large sample, in statistical terms.

Table 4

Size and shape of *T. aestivum* ascospores from B&H, based on measurements of 100 spores (25 ascii with four spores)

No.	*l*	*w*	*Q*
1	**2**	**3**	**4**
1	26	19.5	1.33
2	26.2	18.8	1.39
3	27	19.8	1.36
4	27.1	19.5	1.39
5	27.1	21.7	1.25
6	27.1	20.9	1.3
7	27.2	18.8	1.47
8	27.7	23.9	1.16
9	27.7	20.4	1.36
10	27.8	21.5	1.29
11	27.8	20.5	1.36
12	27.9	17.2	1.62
13	27.9	21.2	1.32
14	28	19.2	1.46
15	28	21.2	1.32
16	28	22.2	1.26
17	28	19.2	1.46
18	28.1	22.5	1.25
19	28.1	17.8	1.58
20	28.2	21	1.34
21	28.2	21	1.34
22	28.2	20.9	1.35
23	28.2	20.5	1.38
24	28.6	23.3	1.23
25	28.6	22.8	1.25
26	28.7	18.7	1.53
27	28.8	23.4	1.23
28	28.8	20.7	1.39
29	28.8	21	1.37
30	28.9	22.5	1.28
31	29	22.4	1.29
32	29	21	1.38
33	29	24	1.21
34	29.4	22.1	1.33
35	29.5	22	1.34
36	29.6	18	1.64
37	29.6	22.7	1.3
38	29.8	21.4	1.39
39	29.8	23.5	1.27
40	29.9	22.4	1.33
41	30	23	1.3
42	30	20.3	1.48
43	30.1	20.2	1.49
44	30.1	23.1	1.3
45	30.2	22.8	1.32
46	30.2	20.9	1.44
47	30.3	21.6	1.4
48	30.3	22.7	1.33
49	30.4	22.6	1.35

Continuation of Table 4

1	2	3	4
50	31	21.8	1.42
51	31	21.3	1.46
52	31	21.2	1.46
53	31	19.8	1.57
54	31	22.6	1.37
55	31	19.4	1.6
56	31	20.2	1.53
57	31	20.2	1.53
58	31	20.2	1.53
59	31	20.9	1.48
60	31	23	1.35
61	31.1	23.4	1.33
62	30.2	20.2	1.49
63	30.2	21.5	1.4
64	30.3	20.5	1.48
65	30.3	21.7	1.4
66	30.4	20	1.52
67	30.4	19.2	1.58
68	30.4	18.7	1.63
69	30.5	20.1	1.45
70	30.5	21.1	1.45
71	30.5	20.5	1.49
72	30.6	21.9	1.4
73	30.6	21.1	1.45
74	30.7	20.8	1.48
75	30.8	19.2	1.6
76	30.8	26.1	1.18
77	31.9	21.7	1.47
78	32	21.3	1.5
79	32	22.9	1.4
80	32	21.2	1.51
81	32.1	20.5	1.57
82	32.6	21.8	1.49
83	32.7	23.3	1.4
84	32.7	20	1.63
85	32.8	21.5	1.53
86	32.9	21.7	1.51
87	33	23	1.43
88	33	23.3	1.42
89	33.1	21	1.58
90	33.2	21.6	1.54
91	33.2	21	1.58
92	33.3	22	1.51
93	33.3	21	1.59
94	33.4	21.8	1.53
95	33.7	22.2	1.52
96	34	23	1.48
97	34	23	1.48
98	34.3	21.8	1.57
99	34.8	22.7	1.53
100	35.8	23	1.56

Table 5

Comparison of morphological and biometric characteristics of the spores of different origin (climate)

Origin of the material	Spore length (μm)			Spore width (μm)			*Q*
	min.	max.	aver.	min.	max.	aver.	
BiH	26	35.8	30.3	17.2	26.1	21.3	1.42
Croatia[1]	25	32	28.5	23	25	24	1.19
Italy[2]	25	30	27.5	18	22	20	1.37
Italy[3]	24	35	29.5	18	27	22.5	1.31
Turkey[4]	26	28	27	18	20	19	1.42
Turkey[5]	24	48	36	16	38	27	1.33

Notes: [1] – [14]; [2] –[27]; [3] – [24]; [4] – [28]; [5] – [29]

4. Conclusions

Specimens of summer truffles (*T. aestivum*) were found in oak and beech forests on calcareous soils of B&H. Predominant geometric shape of their fruiting bodies is an ellipsoid with an average mass of 44.3 g.

Ascocarps with a larger mass are generally more mature and brown (dark) surfaces predominate in their gleba in relation to white (light) parts. Fully ripened ascocarps are thought to have a richer range of smells and tastes, making them more valuable. But they also have a shorter shelf life, so the larger ones are more demanding for handling and storage.

The spore mass is creamy in immature and yellowish in mature fruiting bodies. Ascii are sessile or with short stalk, containing 1–6 spores, usually 2–4. The average length of spores (l) is 30.3 μm, while the average width of spores (w) is 21.3 μm. The average length of spores of summer truffles, originating from B&H, does not deviate significantly from the length of spores from other climates, which can be said for the average width of spores. When it comes to shape, ellipsoidal form are somewhat more common in our truffle spores than in other climates. Overall, the shape and size of the summer truffle spores native to B&H fit the general picture of this taxon.

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References

[1] Bosnia and Herzegovina – land of diversity. First national Report of Bosnia and Herzegovina for the Convention on biological Diversity (2009). Federal Ministry of Environment and Tourism, 162.

[2] Jukić, N., Omerović, N. (2017). Fungi of the order *Pezizales* in Bosnia and Herzegovina: Endangerment ecology, biogeography. Sarajevo: Amateur Mycological Association, 206.

[3] Ljubojević, S., Marčeta, D., Stupar, V. (2014). Habitat conditions and usability of the hog truffle (*Choiromyces meandriformis* Vitt.) found in Bosnia and Herzegovina. Agrosym 2014. Jahorina, 109–117.

[4] Hamzić Gregorčič, S., Strojnik, L., Potočnik, D., Vogel-Mikuš, K., Jagodic, M., Camin, F. et. al. (2020). Can We Discover Truffle's True Identity? Molecules, 25 (9), 2217. doi: http://doi.org/10.3390/molecules25092217

[5] Strojnik, L., Grebenc, T., Ogrinc, N. (2020). Species and geographic variability in truffle aromas. Food and Chemical Toxicology, 142, 111434. doi: http://doi.org/10.1016/j.fct.2020.111434

[6] Vittadini, C. (1831). Monographia tuberacearum. Mediolani, Ex Ypographia Felicis Rusconi, 88.

[7] Fries, E. (1823): Systema Mycologicvm, Sistens Fvngorvm Ordines, Genera Et Species. Vol. II. Gryphiswaldiae, Svmtibvs Ernesti Mavritii, 620.

[8] Trappe, J. (2001). Taxonomic and nomenclatural problems in the genus *Tuber*. Fédération Française des Trufficulteurs. Aix-en-Provence, 27–29.

[9] Le Tacon, F., Rubini, A., Murat, C., Riccioni, C., Robin, C., Belfiori, B. et. al. (2016). Certainties and uncertainties about the life cycle of the Périgord black truffle (Tuber melanosporum Vittad.). Annals of Forest Science, 73 (1), 105–117. doi: http://doi.org/10.1007/s13595-015-0461-1

[10] Rossbach, M., Stieghorst, C., Polkowska-Motrenko, H., Chajduk, E., Samczyński, Z., Pyszynska, M. et. al. (2019). Elemental analysis of summer truffles Tuber aestivum from Germany. Journal of Radioanalytical and Nuclear Chemistry, 320 (2), 475–483. doi: http://doi.org/10.1007/s10967-019-06485-x

[11] Büntgen, U., Egli, S., Camarero, J. J., Fischer, E. M., Stobbe, U., Kauserud, H. et. al. (2012). Drought-induced decline in Mediterranean truffle harvest. Nature Climate Change, 2 (12), 827–829. doi: http://doi.org/10.1038/nclimate1733

[12] Fischer, C. R., Oliach, D., Bonet, J. A., Colinas, C. (2017). Best Practices for Cultivation of Truffles. Forest Sciences Centre of Catalonia, Solsona, Spain; Yaşama Dair Vakıf, Antalaya, 67.

[13] Stobbe, U., Egli, S., Tegel, W., Peter, M., Sproll, L., Büntgen, U. (2013). Potential and limitations of Burgundy truffle cultivation. Applied Microbiology and Biotechnology, 97 (12), 5215–5224. doi: http://doi.org/10.1007/s00253-013-4956-0

[14] Hrka, J. (1988). Truffles, knowledge – picking – growing – preparing. Zagreb: Mladost, 75.

[15] Montecchi, A, Sarasini, M. (2000). European hypogeous fungi. Vicenza: A. M. B. Fondazione, Centro Studi Micologici, 714.

[16] 8 Things Every Diner Should Know About Truffles (2016). MICHELIN Guide. Available at: https://guide.michelin.com/en/article/dining-in/8-things-every-diner-should-know-about-truffles

[17] Büntgen, U., Bagi, I., Fekete, O., Molinier, V., Peter, M., Splivallo, R. et. al. (2017). New Insights into the Complex Relationship between Weight and Maturity of Burgundy Truffles (Tuber aestivum). PLOS ONE, 12 (1), e0170375. doi: http://doi.org/10.1371/journal.pone.0170375

[18] Bencivenga, M., Baciarelli Falini, L. (2012). Truffle farming guide. Experiences with truffles cultivation in Umbria. Regione Umbria: Universita degli studi di Perugia, 137.

[19] Giangiulio, C. (1995). Truffle farming guide. Regione Abruzzo, Ente Regionale di Sviluppo Agricolo, Avezzano. Unità Operativa Territoriale. Lanciano, 46.

[20] Ljubojević, S. (2016): Solid biofuels: Raw material base – Standards – Production – Environmental considerations. Independent University of Banja Luka, 338.

[21] Colors From Image (2017). HTML Color Codes. Available at: https://html-color-codes.info/colors-from-image/

[22] ColorHexa. Available at: https://www.colorhexa.com

[23] How to choose a good quality black summer truffle (tuber aestivum vittadini) (2020). Pelagaggia Truffles. Available at: https://pelagaggia.com/how-to-choose-a-good-quality-black-summer-truffle-tuber-aestivum-vittadini/

[24] Angelini, P., Bricchi, E., Akhtar, M. S., Properzi, A., Fleming, J. L. E. et. al. (2016). Isolation and Identification of Allelochemicals from Ascocarp of *Tuber* Species. Plant, Soil and Microbes. Vol. 2: Mechanisms and Molecular Interactions. Springer: 225–252. doi: http://doi.org/10.1007/978-3-319-29573-2_11

[25] Castellano, M. A., Trappe, J. M., Maser, Z., Maser, C. (1989): Key to spores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy. Mad River Press Inc., Eureka, 196.

[26] Mello, A., Murat, C., Bonfante, P. (2006). Truffles: much more than a prized and local fungal delicacy. FEMS Microbiology Letters, 260 (1), 1–8. doi: http://doi.org/10.1111/j.1574-6968.2006.00252.x

[27] Molinier, V., Peter, M., Stobbe, U., & Egli, S. (2016). The Burgundy Truffle (Tuber aestivum syn. uncinatum): A Truffle Species with a Wide Habitat Range over Europe. True Truffle (Tuber Spp.) in the World. Springer, 33–47. doi: http://doi.org/10.1007/978-3-319-31436-5_3

[28] Türkoğlu, A., Castellano, M. A., Trappe, J. M.,& Yaratanakul Güngör, M. (2015). Turkish truffles I: 18 new records for Turkey. Turkish Journal of Botany, 39, 359–376. doi: http://doi.org/10.3906/bot-1406-42

[29] Alkan, S., Aktaş, S., Kaşık, G. (2018). Tuber Species in Turkey and A New Locality for Tuber aestivum. Selcuk University Journal of Science Faculty, 44 (1), 25–29.

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