#### ISSN 1678-992X



# Production and marketing of *Tuber floridanum* – ecology and gastronomic value of a recently described truffle species

Joice Aline Freiberg<sup>1</sup>\*<sup>(0)</sup>, Tine Grebenc<sup>2</sup><sup>(0)</sup>, Lidija Strojnik<sup>3</sup><sup>(0)</sup>, Leonardo Arocha Meireles<sup>1</sup><sup>(0)</sup>, Maximiliano Segundo Escalona Jiménez<sup>4</sup><sup>(0)</sup>, Neila Silvia Pereira dos Santos Richards<sup>4</sup><sup>(0)</sup>, Nives Ogrinc<sup>3</sup><sup>(0)</sup>, Zaida Inês Antoniolli<sup>1</sup><sup>(0)</sup>

<sup>1</sup>Universidade Federal de Santa Maria/Centro de Ciências Rurais – Depto. de Solos, Av. Roraima, 1000 – 97105-900 – Santa Maria, RS – Brasil.

<sup>2</sup>Slovenian Forestry Institute – Dept. of Forest Physiology and Genetics, Večna pot 2 – 1000 – Ljubljana – Slovenia.
<sup>3</sup>Jožef Stefan Institute – Dept. of Environmental Sciences, Jamova cesta 39 – 1000 – Ljubljana – Slovenia.
<sup>4</sup>Universidade Federal de Santa Maria/Centro de Ciências Rurais – Depto. de Tecnologia e Ciência dos Alimentos, Av. Roraima, 1000 – 97105-900 – Santa Maria, RS – Brasil.
\*Corresponding author <jaf.freiberg@gmail.com>

Edited by: Fernando Dini Andreote

Received May 17, 2022 Accepted September 22, 2022 **ABSTRACT**: *Tuber floridanum* is the first truffle species reported in Brazil and little is known about its nutritional properties and ecology. Therefore, this study aimed to (1) determine *T. floridanum* aroma and nutritional properties and (2) analyze cultivation and fruiting conditions, including potential crop pests and market value. Fruiting bodies of *T. floridanum* showed a slightly ellipsoid shape and smooth to shallow groovy surface. The mass of ascocarps ranged from 0.13 g to 15.95 g, and the truffle was composed of 71 % carbohydrates, 19 % protein, 5 % ash, and 5 % of fat. Octan-3-one, 1-methoxy-3-methyl-butane, and 1-methoxy-2-methyl-butane determined *T. floridanum* characteristic aroma. Soil properties were also specific for this truffle, differing from optimal soils where commercial truffles are produced. In addition, the occurrence of earwigs (Anisolabididae, Anisolabidinae) feeding on truffles (26 % of all ascocarps) in pecan orchards may reduce truffle quality and cause financial losses. *T. floridanum* aroma has a unique composition, completely different from any commercial and non-commercial truffle species analyzed so far. Soil conditions in pecan orchards were also specific for truffle fruiting. Further studies should elucidate other ecological conditions for the co-production of *T. floridanum* with pecan. **Keywords**: aroma profile, mycophagy, nutritional properties, pecan, soil

# Introduction

*Tuber floridanum* Grupe, Sulzbacher & ME Smith is a recently described truffle species from the *Maculatum* clade, which encompasses white truffles characterized by small and light brown to tan ascocarps. The species is native to North America, but our study group first discovered its mature sporocarps in Rio Grande do Sul, Brazil (Grupe et al., 2018). In both the southeastern United States and southern Brazil, collections are known solely from wild or cultivated pecan habitats (Grupe et al., 2018; Sulzbacher et al., 2019).

All known truffle species are ectomycorrhizal (Rinaldi et al., 2008) or can form ectendomycorrhizal or endophytic relationships under specific conditions. Truffles form mycorrhiza with trees and shrubs and rarely interact with non-ectomycorrhizal plants in natural environments (Ori et al., 2020; Schneider-Maunoury et al., 2020; Nahberger et al., 2021). Besides vital mycorrhizal plant partners, most truffle species require specific soil conditions to prosper and fruit, such as neutral to alkaline pH (Hall et al., 2007; Ge et al., 2017), high concentration of available calcium, and low organic matter contents (Hall et al., 2007; Zambonelli et al., 2017). There is little information on T. floridanum, although this species started to be commercialized as Sapucay truffle; thus, there is growing interest for T. floridanum cultivation in truffle orchards.

Due to their high cultural and gastronomic prestige, various truffle species have well established ecology and are appreciated worldwide for their high nutritional value, bioactive compounds, and prestigious aroma (Patel et al., 2017; Yan et al., 2017; Lee et al., 2020). Scientific background data is required for the adequate cultivation and commercialization of *T. floridanum*. Olfactory (aroma) and nutrition characterization are necessary for culinary purposes and to add market value to the species, as it has no history of gastronomic applications. Further, minimum ecological requirements are needed to cultivate *T. floridanum* in controlled environments (orchards) or in natural stands to allow for cultivation and economic viability. Therefore, this study aimed (1) to determine *T. floridanum* aroma and nutritional properties and (2) to analyze conditions for cultivation and fruiting, including investigating potential pests that affect truffle production and market value.

# **Materials and Methods**

#### Sampling

Truffle samples were collected, as described in Castellano et al. (2004), by manually removing cover crops and racking the topsoil in pecan orchards in Rio Grande do Sul State, Brazil, between the years 2018-2020. Truffles were collected in two producing commercial pecan orchards in the municipality of Cachoeira do Sul, Rio Grande do Sul State, Brazil (29°56'11.79" S, 52°47'23.71" W, 116 m altitude; 30°05'03.61" S, 52°46'24.32" W, 86 m altitude). Orchard A, with regular and abundant fruiting (2018-2020), and Orchard B, with little presence of sporocarps in 2020 (three sporocarps < 2 g each). The collections were performed during the summer, from Nov to Jan, at least twice a month. The fruiting bodies from Orchard



A were collected, cleaned under tap water to remove soil particles and were either: a) used fresh for size and weight measurements, aroma analyses, and excision of truffle dwelling insects); b) frozen at -20 °C for molecular identification; or c) freezedried for subsequent nutritional analysis and storage in herbarium collection.

# Nutritional composition, aroma profile, and molecular analyses

The macro-Kjeldahl method (N  $\times$  4.38) was used to estimate the crude protein (CP) content (AOAC, 2000). The total crude fat was determined by extraction with chloroform-methanol-water as solvents (Bligh and Dyer, 1959), and the ash content was determined by incineration at 600  $\pm$  15 °C (AOAC, 2000). Total carbohydrates were calculated by difference (Yan et al., 2017). The analyses were performed in triplicate for each sampling season, and the nutritional value was expressed in percentage for each component.

The aroma profile was determined on 33 individual sporocarps collected in 2020 from Orchard A. The ripeness of asci ranged from 19% - 90% (Standard Deviation (SD) = 13 %; average 76 %), and the weight of sporocarps ranged from 0.36 g – 3.77 g (SD = 0.97 g; average 1.41 g). Sample preparation for aroma profile, gas chromatography-mass spectrometry (GC-MS), and data analyses was performed as described by Strojnik et al. (2020).

Molecular identification of sporocarps was carried out through nrDNA ITS sequencing following the methodology in Grupe et al. (2018). The complete nrDNA internal transcribed spacer (ITS) sequence was amplified with the primer pair ITS1f/ITS4. PCR conditions followed the protocol of Nahberger et al. (2021). DNA was sequenced with the same primers used for PCR.

#### Ecological aspects - soil properties and mycophagy

Topsoil samples (0-20 cm) were sampled in nine productive orchards in 2020 (nine orchards × ten composite samples = 90 samples) for the following soil chemical properties analysis: pH, P, K, Ca, Mg, Zn, Cu, B, S, Al, contents of clay and organic matter (OM) (Teixeira et al., 2017). Specimens of truffle-dwelling invertebrates were collected from ascocarps surface or bore holes in the ascocarps (Lilleskov and Bruns, 2005) conserved in 80 % ethanol and identified in the Laboratory of Entomology at the Universidade de Santa Cruz do Sul, Santa Cruz do Sul, Rio Grande do Sul, Brazil. Invertebrate specimens were checked for spores in the digestive tract to confirm active feeding on the truffle. Preyed vs. intact sporocarps were quantified and weighed.

#### Data analysis

Data on the nutritional content of ascocarps (n = 9) were submitted to the Kruskal-Wallis (p < 0.05) non-parametric test to compare differences between the

years of truffles sampling. Soil properties were shown as average  $\pm$  standard deviation. All analyses were performed in R (R Core Team, 2021, version 4.1.1).

#### Results

#### Nutritional composition and aroma profile

Hunting for truffles requires special skills and preferably with the aid of trained animals (dogs) and knowledge of the ecology (including phenology) of truffles. The regular and systematic searches for truffles in pecan orchards showed a regular production in Orchard A and an occasional production in Orchard B. Ripened truffles were mainly collected in Nov 2018, Dec 2019, and Dec 2020, with rare or unripe specimens outside these periods. Reference ascocarps are deposited under the codes SMDB 20340, SMDB 20338, and SMDB 20339 at the SMDB Herbarium (Santa Maria - Department of Biology) of the Universidade Federal de Santa Maria (GenBank accession numbers: OP132942 to OP132946). Length  $\times$  width of truffles collected from 241 ascocarps in 2019 ranged from 7.9 mm  $\times$  6.8 mm to 37.4 mm  $\times$  33.5 mm, with average Q-value = 1.17 (SD = 0.13), indicating a roundish to slightly ellipsoid shape, while the mass of ascocarps ranged from 0.13 g to 15.95.

*Tuber floridanum* was, on average, composed of 71 % carbohydrates, 19 % protein, 5 % ash remaining, and 5 % of fat (Figure 1). No differences were observed



Figure 1 – Carbohydrates, protein, ash remaining, and fat (%) of *Tuber floridanum* collected in three productive seasons (2018-2020) in a pecan orchard (Cachoeira do Sul, Rio Grande do Sul State, Brazil).

between years according to the Kruskal-Wallis test (p = 0.3679 carbohydrates, p = 0.7897 protein, p = 0.5647 ash, and p = 0.068 fat). The volatile compounds mixture (aroma) of *T. floridanum* (GenBank accession numbers: OM212436 to OM212445), as expected, differed from other commercial and non-commercial species commonly collected in Europe (Table 1). The three most common volatile organic compounds found were octan-3-one, with 32.9 average area per cent in GC-MS, followed by 1-methoxy-3-methyl-butane (19.1 average area per cent) and 1-methoxy-2-methyl-butane (10.1 average area per cent).

#### Ecological aspects - soil properties and mycophagy

We analyzed the soil chemical and physical properties of nine pecan orchards (Table 2). Nutrients and the pH varied significantly between plantations; however, no relation between truffles producing and nonproducing orchards was observed. Some properties in the producing orchards showed similar pH levels and nutrient concentrations, such as P, Cu, B, Al, Ca, and Mg. Potassium and S were higher in Orchard A, while Zn was higher in Orchard B.

Regarding mycophagy, *T. floridanum* is susceptible to the attack of invertebrates, which were found preying on truffles. Among the 241 ascocarps collected, 26 % were damaged by earwigs. The earwig species belonging to the Anisolabididae family, subfamily Anisolabidinae, order Dermaptera, was the main truffle fungivores (Figure 2A). No ascocarps were partially or severely damaged (eaten) by earwigs (Figures 2B and 2C), with spores also occurring in their digestive tract (Figure 2D).

### **Discussion**

*T. floridanum* showed a roundish to slightly ellipsoid shape, common in most truffles (Trappe et al., 2009). In addition, the surface was smooth to shallowly groovy, facilitating ascocarp cleaning and its characterization as a suitable shape for trading. Ascocarp weight is the main parameter that determines the market price of each truffle species. *T. floridanum* weight was within the range > 2 g to < 750 g, and the average mass of 35 g reported for

**Table 1** – Comparison of relative areas per cent of *Tuber floridanum* volatile organic compounds to the previous reported volatile organic compounds relative areas per cent by Strojnik et al. (2020) for truffles collected in Europe.

Volatile organic compounds	T. flo	T. aes	T. aes*	T. bru	T. exc	T. ind	T. mac	T. mag	T. mel	T. mes	T. ruf
1-methoxy-3-methylbenzene	nd	3.7	5.7	28.3	37.9	44.5	10.6	0.3	4.2	69.5	17.4
bis(methylsulfanyl)methane	nd	nd	nd	nd	nd	nd	nd	67.8	nd	nd	nd
butan-2-one	nd	53.3	10.4	0.6	0.4	0.3	0.2	nd	1.3	0.3	0.8
(E)-1-methylsulfanylprop-1-ene	nd	0.2	nd	nd	29.6	nd	27	nd	nd	0.3	nd
2-methylbutanenitrile	nd	nd	nd	nd	nd	nd	0.1	nd	nd	nd	21.9
1-methylsulfanylpropane	nd	0.1	nd	nd	0.4	nd	22.1	nd	nd	nd	nd
2-methylbutan-1-ol	nd	4.7	1.7	0.4	nd	nd	0.6	1.2	21.9	nd	3.2
methylsulfanylmethane	6.2	18.9	3.8	4.5	4.9	4.3	1.3	14.6	11.7	1.2	8.2
butan-2-yl formate	0.1	nd	nd	7	nd	11.4	0.2	nd	15.7	nd	2.2
oct-1-en-3-o	2.6	0.9	47.4	11.2	2.4	0.2	4.7	nd	2.0	9.6	3.1
3-methylbutan-1-ol	1.3	nd	nd	nd	1.8	6.5	nd	0.2	nd	nd	5.4
octan-3-one	32.9	2.6	16.1	8.8	2.0	0.2	7.3	0.3	4.2	4.2	5.3
1,4-dimethoxybenzene	nd	0.1	nd	12.1	2.8	nd	2.7	0.1	0.1	0.9	0.7
butan-2-ol	nd	6.3	1.9	0.1	nd	nd	nd	nd	nd	nd	0.3
2-methylpropyl formate	0.8	nd	nd	0.9	nd	nd	nd	nd	1.0	nd	1.4
2-methylbutanal	nd	0.1	nd	0.8	nd	nd	nd	0.1	8.4	nd	0.4
undec-1-ene	nd	0.1	nd	2.9	1.8	nd	0.4	1.8	nd	nd	nd
(Z)-1-methylsulfanylprop-1-ene	nd	nd	nd	nd	6.7	nd	8.0	nd	nd	0.2	nd
1,4-dimethoxy-2-MB* *	nd	nd	nd	1.4	0.1	1.2	1.6	nd	0.1	7.8	0.1
anisole	0.1	nd	nd	3.4	0.7	1.4	0.8	nd	7.1	nd	0.2
3-methylbutanal	4.5	1.2	1.6	0.6	0.8	2.8	nd	1.5	6.5	nd	1.6
2-nitropentane	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.9
2-bromo-2-methylbutane	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.3
(methyldisulfanyl)methane	nd	nd	nd	0.1	0.1	nd	0.2	2.8	0.2	nd	nd
Butane, 1-methoxy-3-methyl-	19.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Butane, 1-methoxy-2-methyl-	10.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Propane, 1-methoxy-2-methyl-	6.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Other	16.0	7.7	11.5	17.0	7.6	27.2	12.3	9.4	15.6	6.0	21.7

nd = not detected; \*Potential Tuber mesentericum or a cryptic species in Tuber aestivum agg. (Marozzi et al., 2020); \*\*1,4-dimethoxy-2-MBA methylbenzene. Tuber floridanum (T. flo), T. aestivum (T. aes), T. brumale (T. bru), T. excavatum (T. exc), T. indicum (T. ind), T. macrosporum (T. mac), T. magnatum (T. mag), T. melanosporum (T. mel), T. melanosporum (T. mel), T. mesentericum (T. mes) and T. rufum (T. ruf).

Table 2 - Soil prop(	erties in pecar	n orchards wit	th presence	or absence of fr	uit bodies of Tu	ber floridanum,	in Rio Grande c	lo Sul state, Br	azil.			
Chemical Properties	Clay	рН Н <sub>2</sub> О	MO	٩	Х	Zn	Cu	В	S	AI	Ca Mg	
	%		%			mg dm	-3				- cmol <sub>c</sub> dm <sup>-3</sup>	
Pecan orchard – Prese	snce of fruit bo	dies of Tuber fl	loridanum									
Cachoeira do Sul (A)	$25.9 \pm 1.29$	$5.51 \pm 0.19$	$1.57 \pm 0.29$	$55.86 \pm 19.33$	$218.8 \pm 26.06$	$3.833 \pm 1.13$	$2.059 \pm 0.16$	0.354± 0.10 7	.31 ± 2.83	0.032 ± 0.05	4.665 ± 0.47 2.115 ±	0.22
Cachoeira do Sul (B)	$18.8 \pm 2.20$	$5.78 \pm 0.40$	$1.20 \pm 0.12$	$52.66 \pm 17.94$	89.6 ± 16.02	9.809 ± 4.32	$1.932 \pm 0.27$ (	0.288 ± 0.07 3	8.86 ± 0.99	0.03 ± 0.06	$4.511 \pm 0.85 1.630 \pm$	0.25
Pecan orchard – Abse	nce of fruit bod	lies of Tuber flc	oridanum									
Catuípe	$55.6 \pm 3.57$	$5.18 \pm 0.18$	$2.12 \pm 0.31$	$12.37 \pm 5.34$	221.2 ± 37.57	$1.274 \pm 0.28$	$4.879 \pm 0.51$ (	).265 ± 0.05 15	.89 ± 3.03	$0.19 \pm 0.09$	4.968 ± 0.48 2.376 ±	0.26
ljuí	$28.0 \pm 8.46$	$5.51 \pm 0.19$	$1.75 \pm 0.55$	$5.79 \pm 2.65$	268.4 ± 72.97	8.097 ± 2.72	9.997 ± 1.88 (	).338 ± 0.08 13	.95 ± 2.14	$0.053 \pm 0.05$	13.307 ± 4.77 3.894 ±	1.57
Anta Gorda	$15.2 \pm 1.93$	5.76 ± 0.32	$3.98 \pm 1.10$	94.85 ± 128.40	) 313.2 ± 82.78	36.152 ± 13.10	9.31 ± 8.89 (	0.298 ± 0.06 1	.3.7 ± 4.14	0.02 ± 0.04	17.249 ± 4.88 4.229 ±	0.90
Santa Maria	$14.3 \pm 1.57$	$5.71 \pm 0.45$	$1.38 \pm 0.13$	6.98 ± 3.66	$118.8 \pm 29.82$	$5.25 \pm 3.61$	2.234 ± 0.17 (	$0.314 \pm 0.03$ 4	1.72 ± 1.91	$0.055 \pm 0.09$	2.886 ± 0.64 1.483 ±	0.32
Cachoeira do Sul (C)	$13.6 \pm 3.84$	$5.97 \pm 0.29$	$1.67 \pm 0.40$	$350.36 \pm 21.42$	$421.6 \pm 95.0$	$18.407 \pm 10.71$	$1.395 \pm 0.13$ (	).489 ± 0.14 23	$3.01 \pm 11.16$	$0.004 \pm 0.01$	6.600 ± 1.47 2.095 ±	0.34
Cachoeira do Sul (D)	$20.6 \pm 3.24$	$4.9 \pm 0.18$	2.76 ± 0.87	$37.15 \pm 19.46$	$251.2 \pm 61.41$	$6.636 \pm 3.60$	$1.991 \pm 0.22$ (	0.303 ± 0.08 17	'.75 ± 3.04	$0.531 \pm 0.27$	6.904 ± 2.89 3.059 ±	1.30
Pantano Grande	$12.9 \pm 1.79$	$5.77 \pm 0.37$	$1.47 \pm 0.23$	9.42 ± 1.76	$45.8 \pm 11.17$	$2.027 \pm 0.90$	$3.537 \pm 0.75$	0.19 ± 0.08 8	3.12 ± 1.08	0.029 ± 0.06	5.227 ± 1.33 1.811 ±	0.39
OM = Organic matter.												

commercial black truffle species, such as *Tuber aestivum* (Le Tacon et al., 2013; Büntgen et al., 2017). Weights and sizes of truffles may vary significantly; however, these variations of sporocarps of the T. floridanum collected were on, average lower than those observed for most commercial black truffles; nevertheless, within the range of Tuber brumale (Montecchi and Sarasini, 2000) and the white truffle species Tuber borchii (Benucci et al., 2012). Despite the smaller average size and fresh weight of T. floridanum (241 ascocarps collected in 2019), ascocarps reached up to 134 g in the 2021 harvest, indicating a production potential with dimensions highly valued for commercial truffles.

Values of the nutritional composition of T. floridanum are similar to data from three Asian Truffles (Yan et al., 2017) for which carbohydrates contents ranged from 74 % to 79 %, protein content from 11 % to 15 %, and fat content from 2 % to 3 %. On the other hand, the protein content of European commercial truffle species differed from that in T. floridanum with, 20 % to 24 % in T. magnatum, 6 % to 13 % in T. borchii, 7 % to 9 % in T. melanosporum, and 11 % to 13 % in T. aestivum (Saltarelli et al., 2008). Truffles are primarily used in dishes as a spice in small quantities (1 g - 20 g per dish), not as the main ingredient. Therefore, we do not expect that the nutritional composition of T. floridanum plays a significant role in its culinary and commercial value, as opposed to truffle aroma, which is a highly appreciated characteristic.

Truffle aroma is a mixture of volatile organic compounds that, along with other ascocarp-associated microbes (yeast and fungi), contribute significantly to the complex truffle aroma (Vita et al., 2015). The complex mixture of volatiles in truffle is peculiar to the species (Šiškovič et al., 2021), with additional high variation in the quantity of individual volatiles related to truffle origin, soil conditions, and maturity (Splivallo et al., 2012; Culleré et al., 2013; Strojnik et al., 2020). The most common volatile organic compound in T. floridanum (octan-3-one) was present in all other European truffles analyzed (Strojnik et al., 2020) and in Asian T. japonicum (Shimokawa et al., 2020) but never exceeding the average area of 10 %. Therefore, it can be regarded as the dominant aroma component and the "significant scent" of T. floridanum volatile compounds mixture. The other two dominant volatile compounds (1-methoxy-3methyl-butane and 1-methoxy-2-methyl-butane), along with the 1-methoxy-2-methyl-propane, were unique to T. floridanum volatile components and can be regarded as the "distinctive scent", separating this species from all other truffle species analyzed (Strojnik et al., 2020).

The olfactory evaluation of a specialist of T. floridanum ascocarps aroma recognized aromas of butter, grease, sweet, ether, and "general fungi/mouldy". From the gastronomic viewpoint, T. floridanum aroma differs completely from aroma analyzed in commercial and noncommercial truffle species so far, conferring the species an opportunity to fill a gap in this gastronomic niche,



**Figure 2** – Earwig (Anisolabididae, Anisolabidinae) preying on *Tuber floridanum* (bar = 1 cm) (A). Ascocarps partially or severely damaged (eaten) by the earwig (bar = 1 cm) (B, C). *Tuber floridanum* spores from the digestive tract of the earwig (bar = 35  $\mu$ m) (D). Credit: Joice Aline Freiberg.

while allowing chefs to discover new ways of preparing and serving this species in unique and innovative ways.

Regarding soil properties, nutrient composition, the pH, and soil structure are vital parameters determining truffle survival and fruiting in orchards. Orchards for the production of pecan require specific, but not necessarily, truffle-compatible management (Sulzbacher et al., 2019). The orchards studied significantly do not present optimal soils for some of the commercial production of truffles. For example, in soils for *T. melanosporum* production, the pH varies from 7.10 to 8.15 and the clay content ranges from 10 to 45 % (García-Montero et al., 2006; Raglione and Owczarek, 2005). T. aestivum requires soils of better quality and generally has broader ecological requirements, with a pH between 6.3-8.5 (Stobbe et al., 2013). None of the pecan orchards analyzed supports the cultivation of these two species. T. floridanum appears to fruit well also in soils with lower pH when compared to other commercial truffles, as the pH varied between 5.3-5.8 in Orchard A and 5.2-6.4 in Orchard B. The pH has an important influence on the structure of ectomycorrhiza fungi communities, as truffle frequency and abundance increase in soils with a higher pH (Ge et al., 2017). In this sense, Ca is also an essential nutrient for truffles production since it increases soil pH (García-Montero et al., 2006), as well K, Mg, Mn, which are also correlated with pH (Ge et al., 2017).

The macronutrients P and K are not necessary at high concentrations for successful truffle production; however, optimal contents reported for black truffles range between 100-300 mg dm<sup>-3</sup> (Fischer et al., 2017). In our study, we found low contents of Zn and Cu; however, high values of Cu (61.1 mg kg<sup>-1</sup>) and Zn (12.1 mg kg<sup>-1</sup>) were observed in soils of *T. aestivum*, and 9.0 mg kg<sup>-1</sup> of Zn and 17.9 mg kg<sup>-1</sup> Cu in Italian soils of *T. melanosporum* production (Raglione and Owczarek, 2005). In summary, each truffle species has specific requirements and growth limits. There is detailed information on ecological and management requirements for the cultivation of Tuber melanosporum (García-Montero et al., 2006; Fischer et al., 2017) or T. aestivum (Stobbe et al., 2013; Chevalier and Sourzat, 2012); nevertheless, our study provides enough data on T. floridanum ecology for cultivation in orchards or ecologically modified natural pecan stands,

along with previous information (Grupe et al., 2018; Sulzbacher et al., 2019)

In truffle cultivation, interactions between truffles and invertebrate pests can significantly affect the production quantity and quality of ripened ascocarps, drastically decreasing producers' incomes (Rosa-Gruszecka et al., 2017). Chen et al. (2014) investigated earwigs fed on fungi and reported the mycophagy of the fetid mushroom Lysurus mokusin (L.) Fr. (Phallaceae) by the earwig Anisolabis maritima (Bonelli, 1832). In addition to dispersing spores attached to the bodies, the authors found that the primary dispersal mechanism occurs via the intestines (feces), which increases the viability and germination of fungal spores. Moreover, earwigs Chelidurella acanthopygia (Genè, 1832) consume all types of fungal material (spores, hyphae, and fruiting body fragments), as well as Apterygida media (Hagenbach, 1822) that may consume fungi on trees trunks and branches (Kirstová et al., 2019). Therefore, ascocarps become part of the earwig diet at truffle fructification, possibly due to their high abundance and availability as food. In this context, a better view of the temporal dynamics of earwigs preying on truffles may support adopting of appropriate measures to reduce truffle quality loss and financial losses for truffle producers.

In this study, we reported the nutritional properties and aroma profile of *T. floridanum*, soil conditions, and the mycophagy case of earwigs in the truffle. Truffle aroma differed entirely from any commercial and non-commercial truffle species analyzed. Regarding soil properties for cultivation and fruiting, we provide necessary ecology data for *T. floridanum* cultivation in orchards or for modifications of natural pecan stands. These results contribute to the knowledge of *T. floridanum*. Further studies should elucidate soil conditions for truffle production and the co-production of truffles with pecan in Brazil, also investigating the microbiome and the environmental effects on the nutritional properties of this truffle species.

## Acknowledgments

We thank Dr. Köhler A. for determining the earwigs, Dr. Sulzbacher M.A. for providing information on yearly harvest and data quantification for *Tuber* 

floridanum in Brazil. We are grateful to the group of the Laboratory of Soil Biology and Mycrobiology (Universidade Federal de Santa Maria - UFSM) for helping in the field. We appreciate the scholarship the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) provided. We also thank Paralelo 30 from Cachoeira do Sul, Rio Grande do Sul, Brazil, pecan producers and the Instituto Brasileiro de Pecanicultura (IBPecan). This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Project 423477/20188. The study was further supported by the Slovenian Research Agency (Research Programmes P4-0107 and P1-0143, and research projects J4-1766, J4-4547 and J4-3098). Further funding was provided by the REALMed project (ARIMNet2 2014-2017) and ERA-NET coordinated by INRA-France, IAEA project (23362), and by the Slovenian Ministry of Education, Science and Sport (3330-17-500186).

# **Authors' Contributions**

Conceptualization: Freiberg, J.A.; Grebenc, T.; Antoniolli, Z.I. Data curation: Freiberg, J.A.; Strojnik, L. Formal analysis: Freiberg, J.A. Investigation: Freiberg, J.A.; Grebenc, T.; Strojnik, L.; Meireles, L.A.; Jiménez, M.S.E.; Antoniolli, Z.I. Resources: Grebenc, T.; Richards, N.S.P.S.; Ogrinc, N.; Antoniolli, Z.I. Writing-original draft: Freiberg, J.A.; Grebenc, T. Writing-review & editing: Freiberg, J.A.; Grebenc, J.A.; Strojnik, L.; Ogrinc, N.; Antoniolli, Z.I.

# References

- Association of Official Analytical Chemists International [AOAC]. 2000. Official Methods of Analysis. 18ed. AOAC, Gaithersburg, MD, USA.
- Bligh, E.G.; Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917. https://doi.org/10.1139/o59-099
- Benucci, G.M.N.; Bonito, G.; Falini, L.B.; Bencivenga, M. 2012. Mycorrhization of pecan trees (*Carya illinoinensis*) with commercial truffle species: *Tuber aestivum* Vittad. and *Tuber borchii* Vittad. Mycorrhiza 22: 383-392. https://doi.org/10.1007/ s00572-011-0413-z
- Büntgen, U.; Bagi, I.; Fekete, O.; Molinier, V.; Peter, M.; Splivallo, R.; Vahdatzadeh, M.; Richard, F.; Murat, C.; Tegel, W.; Stobbe, U.; Martínez-Peña, F.; Sproll, L.; Hülsmann, L.; Nievergelt, D.; Meier, B.; Egli, S. 2017. New insights into the complex relationship between weight and maturity of Burgundy truffles (*Tuber aestivum*). PLoS One 12: e0170375. https://doi. org/10.1371/journal.pone.0170375
- Castellano, M.A.; Trappe, J.M.; Luoma, D.L. 2004. Sequestrate fungi. p. 197-213. In: Foster, M.S; Mueller, G.M; Bills, G.F., eds. Biodiversity of Fungi: Inventory and Monitoring Methods. Academic Press, Burlington, USA.

- Chen, G.; Zhang, R-R.; Liu, Y.; Sun, W-B. 2014. Spore dispersal of fetid *Lysurus mokusin* by feces of mycophagous insects. Journal of Chemical Ecology 40: 893-899. https://doi.org/10.1007/ s10886-014-0481-6
- Chevalier, G.; Sourzat, P. 2012. Soils and techniques for cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe.
  p. 163-189. In: Zambonelli, A.; Bonito, G., eds. Edible ectomycorrhizal mushrooms. Springer, Berlin, Germany. https://doi.org/10.1007/978-3-642-33823-6\_10
- Culleré, L.; Ferreira, V.; Venturini, M.E.; Marco, P.; Blanco, D. 2013. Potential aromatic compounds as markers to differentiate between *Tuber melanosporum* and *Tuber indicum* truffles. Food Chemistry 141: 105-110. https://doi.org/10.1016/j. foodchem.2013.03.027
- Fischer, C.R.; Oliach, D.; Bonet, J.A.; Colinas, C. 2017. Best Practices for Cultivation of Truffles. Forest Sciences Centre of Catalonia, Solsona, Spain.
- García-Montero, L.G.; Casermeiro, M.A.; Hernando, J.; Hernando, I. 2006. Soil factors that influence the fruiting of *Tuber melanosporum* (black truffle). Australian Journal of Soil Research 44: 731-738. https://doi.org/10.1071/SR06046
- Ge, Z.-W.; Brenneman, T.; Bonito, G.; Smith, M.E. 2017. Soil pH and mineral nutrients strongly influence truffles and other ectomycorrhizal fungi associated with commercial pecans (*Carya illinoinensis*). Plant and Soil 418: 493-505. https://doi. org/10.1007/s11104-017-3312-z
- Grupe, A.C.; Sulzbacher, M.A.; Grebenc, T.; Healy, R.; Bonito, G.; Smith, M.E. 2018. *Tuber brennemanii* and *Tuber floridanum*: two new *Tuber* species are among the most commonly detected ectomycorrhizal taxa within commercial pecan (*Carya illinoinensis*) orchards. Mycologia 110: 780-790. https://doi.org/ 10.1080/00275514.2018.1490121
- Hall, I.R.; Brown, G.T.; Zambonelli, A. 2007. Taming the Truffle: The History, Lore and Science of the Ultimate Mushroom. Timber, Portland, OR, USA.
- Kirstová, M.; Pyszko, P.; Kočárek, P. 2019. Factors influencing microhabitat selection and food preference of tree-dwelling earwigs (Dermaptera) in a temperate floodplain forest. Bulletin of Entomological Research 109: 54-61. https://doi.org/10.1017/ S0007485318000147
- Le Tacon, F.; Zeller, B.; Plain, C.; Hossann, C.; Bréchet, C.; Robin, C. 2013. Carbon transfer from the host to *Tuber melanosporum* mycorrhizas and ascocarps followed using a 13C pulse-labeling technique. PLoS One 8: e64626. https://doi.org/10.1371/ journal.pone.0064626
- Lee, H.; Nam, K.; Zahra, Z.; Farooqi, M.Q.U. 2020. Potentials of truffles in nutritional and medicinal applications: a review. Fungal Biology and Biotechnology 7: 9. https://doi.org/10.1186/ s40694-020-00097-x
- Lilleskov, E.A.; Bruns, T.D. 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97: 762-769. https://doi.org/10.1080/1557253 6.2006.11832767
- Montecchi, A.; Sarasini, M. 2000. Hypogeous fungi of Europa = Funghi Ipogei D'Europa. Fondazione Centro Studi Micologici, Vicenza, Italy (in Italian).
- Marozzi, G.; Benucci, G.M.N.; Suriano, E.; Sitta, N.; Raggi, L.; Lancioni, H.; Baciarelli Falini, L.; Albertini, E.; Donnini,

D. 2020. *Tuber mesentericum* and *Tuber aestivum* truffles: new insights based on morphological and phylogenetic analyses. Diversity 12: 349. https://doi.org/10.3390/d12090349

- Nahberger, T.U.; Benucci, G.M.N.; Kraigher, H.; Grebenc, T. 2021. Effect of earthworms on mycorrhization, root morphology and biomass of silver fir seedlings inoculated with black summer truffle (*Tuber aestivum* Vittad.). Scientific Reports 11: 6167. https://doi.org/10.1038/s41598-021-85497-8
- Ori, F.; Leonardi, M.; Faccio, A.; Sillo, F.; Iotti, M.; Pacioni, G.; Balestrini, R. 2020. Synthesis and ultrastructural observation of arbutoid mycorrhizae of black truffles (*Tuber melanosporum* and *T. aestivum*). Mycorrhiza 30: 715-723. https://doi.org/10.1007/ s00572-020-00985-5
- Patel, S.; Rauf, A.; Khan, H.; Khalid, S.; Mubarak, M.S. 2017. Potential health benefits of natural products derived from truffles: a review. Trends in Food Science & Technology 70: 1-8. https://doi.org/10.1016/j.tifs.2017.09.009
- Raglione, M.; Owczarek, M. 2005. The soils of natural environments for growth of truffles in Italy. Mycologia Balcanica 2: 209-216. https://doi.org/10.5281/zenodo.2547123
- Rinaldi, A.C.; Comandini, O.; Kuyper, T.W. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity 33: 1-45.
- Rosa-Gruszecka, A.; Gange, A.C.; Harvey, D.J.; Jaworski, T.; Hilszczański, J.; Plewa, R.; Konwerski, S.; Hilszcza-ska, D. 2017. Insect-truffle interactions: potential threats to emerging industries? Fungal Ecology 25: 59-63. https://doi.org/10.1016/j. funeco.2016.10.004
- Saltarelli, R.; Ceccaroli, P.; Cesari, P.; Barbieri. E.; Stocchi, V. 2008. Effect of storage on biochemical and microbiological parameters of edible truffle species. Food Chemistry 109: 8-16. https://doi.org/10.1016/j.foodchem.2007.11.075
- Schneider-Maunoury, L.; Deveau, A.; Moreno, M, Todesco, F.; Belmondo, S.; Murat, C.; Courty, P.; Jąkalski, M.; Selosse, M. 2020. Two ectomycorrhizal truffles, *Tuber melanosporum* and *T. aestivum*, endophytically colonise roots of non-ectomycorrhizal plants in natural environments. New Phytologist 225: 2542-2556. https://doi.org/10.1111/nph.16321
- Shimokawa, T.; Kinoshita, A.; Kusumoto, N.; Nakano, S.; Nakamura, N.; Yamanaka, T. 2020. Component features, odor-active volatiles, and acute oral toxicity of novel whitecolored truffle *Tuber japonicum* native to Japan. Food Science & Nutrition 8: 410-418. https://doi.org/10.1002/fsn3.1325
- Šiškovič, N.; Strojnik, L.; Grebenc, T.; Vidrih, R.; Ogrinc, N. 2021. Differentiation between species and regional origin of fresh and freeze-dried truffles according to their volatile profiles. Food Control 123: 107698. https://doi.org/10.1016/j. foodcont.2020.107698

- Splivallo, R.; Valdez, N.; Kirchhoff, N.; Ona, M.C.; Schmidt, J.; Feussner, I.; Karlovsky, P. 2012. Intraspecific genotypic variability determines concentrations of key truffle volatiles. New Phytologist 194: 823-835. https://doi.org/10.1111/j.1469-8137.2012.04077.x
- 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: 5215-5224. https://doi.org/10.1007/s00253-013-4956-0
- Strojnik, L.; Grebenc, T.; Ogrinc, N. 2020. Species and geographic variability in truffle aromas. Food and Chemical Toxicology 142: 111434. https://doi.org/10.1016/j.fct.2020.111434
- Sulzbacher, M.A.; Hamann, J.J.; Fronza, D.; Jacques, R.J.S.; Giachini, A.J.; Grebenc, T.; Antoniolli, Z.I. 2019. Ectomycorrhizal fungi in pecan orchards and the potential of truffle cultivation in Brazil. Ciência Florestal 29: 975-987 (in Portuguese, with abstract in English). https://doi. org/10.5902/1980509827581
- Teixeira, P.C.; Donagemma, G.K.; Fontana, A.; Teixeira, W.G. 2017. Manual of Methods of Soil Analysis = Manual de Métodos de Análise de Solo. 3ed. Embrapa, Brasília, DF, Brazil (in Portuguese).
- Trappe, J.M.; Molina, R.; Luoma, D.L.; Cázares, E.; Pilz, D.; Smith, J.E.; Castellano, M.A.; Miller, S.L.; Trappe, M.J. 2009. Diversity, ecology, and conservation of truffle fungi in forests of the Pacific Northwest. USDA-Forest Service/Pacific Northwest Research Station, Portland, OR, USA. (General Technical Report) (PNW-GTR-772). https://doi.org/10.2737/ PNW-GTR-772
- Vita, F.; Taiti, C.; Pompeiano, A.; Bazihizina, N.; Lucarotti, V.; Mancuso, S.; Alpi, A. 2015. Volatile organic compounds in truffle (*Tuber magnatum* Pico): comparison of samples from different regions of Italy and from different seasons. Scientific Reports 5: 12629. https://doi.org/10.1038/srep12629
- Yan, X.; Wang, Y.; Sang, X.; Fan, L. 2017. Nutritional value, chemical composition and antioxidant activity of three *Tuber* species from China. AMB Express 7: 136. https://doi. org/10.1186/s13568-017-0431-0
- Zambonelli, A.; Leonardi, P.; Iotti, M.; Hall, I. 2017. Ecological and genetic advances in the cultivation of *Tuber* spp. Revista Fitotecnia Mexicana 40: 371-377. https://doi.org/10.35196/ rfm.2017.4.371-377