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ORIGINAL ARTICLE

Persistence of *Tuber melanosporum* in truffle orchards in North Carolina, USA

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Abstract

A survey was conducted to determine the persistence of mycorrhization by *Tuber melanosporum* in truffle orchards established with European and American species of oak and common hazel trees in North Carolina. The trees had reportedly been inoculated and colonized by *T. melanosporum* prior to planting. Root samples were collected from 95 trees among seven orchards in 2015 and roots were analyzed by morphology and quantitative PCR. Samples that tested negative for *T. melanosporum* or where ectomycorrhizal morphology was not observed were analyzed by sequencing to identify the mycorrhizal fungal symbiont present. The presence of *T. melanosporum* was detected in all seven orchards. In six orchards, *T. melanosporum* was detected on all trees, but in only two of fifteen trees in one orchard. Other species of *Tuber* including *T. brennemanii*, *T. canaliculatum*, and *T. lyonii*, species of *Scleroderma*, and members of the Pezizales were also detected by sequence analysis. Sporocarps of *T. aestivum* and *T. brumale* were found in 2017 and 2018 in separate orchards in North Carolina after the survey was conducted. Overall, results indicate that *T. melanosporum* has persisted in truffle orchards sampled in North Carolina. Indigenous and contaminating fungal species, including *Tuber* species, were also detected and present a challenge to the truffle industry in North Carolina.

Keywords European black winter truffle · Ectomycorrhizae · *Tuber melanosporum* · *Tuber* species

Introduction

The European black winter truffle, also referred to as the black Périgord truffle and the French black truffle (*Tuber melanosporum* Vittad.), is one of the most valuable truffles in the world. It is a fungus that grows in an ectomycorrhizal association with host trees, including hazelnut (*Corylus avellana* L.) (Palenzona 1969, Pinkas et al. 2000) and several species of *Quercus* (Boutekrapt et al. 1990; Chevalier and Grente 1979; Michaels 1982; Rahma 2013), producing an edible ascocarp (truffle). *Tuber melanosporum* is native to France, Italy, and Spain where it has been harvested from the wild for hundreds of years (Hall et al. 2007). As wild populations in Europe have declined (Hall et al. 2007) and culinary demand for the truffle has grown, *T. melanosporum* is now widely cultivated within and outside its native range including in Australia, Canada, Chile, New Zealand, South Africa, and the United States (Chen et al. 2016; Reyna and Garcia-Barreda 2014) to meet demand and as a financially lucrative crop.

Australia has been successful in producing European black winter truffles for commercial sale (Lefevre 2012). However, production in commercial quantities in North America has been challenging to achieve and is possibly related to incorrect soil type and pH, lack of seedling certification, genotypic diversity of the fungus, non-favorable climate, and plant host disease (Lefevre 2012; Rubini et al. 2012). Although successful production practices are well documented in Europe (Chevalier and Sourzat 2012), and truffle producers in North America follow these same practices, the success rate is not the same. Successful management practices from one region do not guarantee success in another region (Reyna and Garcia-Barreda 2014) and must often be modified.

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Over the past several decades, hundreds of truffle orchards have been established in the east coast of the USA with over 200 in North Carolina (NC) alone (North American Truffle Growers Association 2020a). Although some orchards have produced truffles (O'Neill 2007, North American Truffle Growers Association 2020b), many orchards report low to no yields (Hall et al. 2007) and are not producing at a sustainable level of commercial productivity. Although climatic conditions in NC are similar to production areas in Europe (Lefevre 2012), the native soil pH and soil textures vary distinctly from production areas in Spain and France (Chevalier and Sourzat 2012; Web Soil Survey 2018). This adds to the complexity of understanding the reproductive biology of *T. melanosporum* in the United States.

Many NC truffle growers had been maintaining their orchards for 5 or more years and questioned whether their orchard trees would ever produce truffles. They asked for a practical and affordable method to determine if their trees were still colonized by *T. melanosporum* so they could decide whether they should continue to invest in orchard management. It was also postulated that other ectomycorrhizae may be present that could be competing with the *T. melanosporum*, but they did not know how to determine that either. Thus, the objectives of this research were to (1) evaluate the presence, or lack thereof, of *T. melanosporum* root colonization in select orchards in NC and (2) determine, which, if any, ectomycorrhizal fungi were present in *T. melanosporum* orchards. The purpose of this study was driven by the orchard producers in NC and supported by limited external funding; therefore, the scope of the project was limited to the detection of *T. melanosporum* and other ectomycorrhizal fungi.

Materials and methods

Orchards included in this study were planted by individual growers and may have had different management strategies. To the best of our knowledge, all trees included in this study were produced in and purchased from one of two nurseries and had been inoculated with *T. melanosporum*. However, trees were not evaluated prior to planting, so the amount of colonization or any other factors prior to planting cannot be confirmed. Host trees in this study included common hazelnut (filbert) (*Corylus avellana* L.), English oak (*Quercus robur* [Ten.] A. DC.), scrub oak (*Q. ilicifolia* Wangenh.), holly oak (*Q. ilex* L.), downy oak (*Q. pubescens* Willd.), Turkish oak (*Q. cerris* L.), and *Quercus* sp. Trees were planted in orchards at various densities ranging from 370 trees/ha to over 1100 trees/ha. High tree density plantings are common in North American truffle orchards, especially those planted with filberts.

Truffle orchards were selected based on age and the owners' willingness to participate. All orchards are in NC and the location of each orchard is listed by county in Table 1. Two orchards, C and D, had been known to produce ascocarps (truffles) of *T. melanosporum* in the past. Three orchards, A, E, and G, were 5 years old or younger and were not expected to produce truffles yet because of their young age. Two orchards, B and F, were of the age to produce (greater than 5 years old), but had not yet been known to produce truffles. Orchard G had been established as a research orchard at a research station in Waynesville, NC. Although the management of each orchard was distinct, in general, lime had been applied to the soil in each orchard at some stage(s) to raise the soil pH to be more conducive to *T. melanosporum* colonization and less conducive to native mycorrhizae. Soil pH is reported in Table 1 if the information was provided by the grower.

All orchards were sampled between October and December 2015. Each orchard was arbitrarily divided into four quadrants and 10 to 18 representative trees in total were selected for sampling based on the size of the orchard. If more than one tree species was present, attempts were made to sample from at least one representative from each tree species. To collect root tips, soil samples (2.54-cm diameter, 15 to 30-cm deep) were collected using a soil probe (Oakfield Apparatus, Oakfield, WI, USA). One to three soil cores were collected on each of four cardinal points of each tree sampled for a total sample of approximately 1 L of soil including roots from each tree. Root samples were maintained within the collected soil at 4–5 °C until processed.

In the laboratory, roots were sieved to remove larger soil particles. Care was taken to avoid damaging roots. Sieved roots were soaked in sterile distilled water for 3–5 min and rinsed, and the process was repeated until roots were adequately clean for microscopic observation. Descriptions of several *Tuber* species (*T. aestivum*, *T. borchii*, *T. brumale*, and *T. melanosporum*) were used as references in an attempt to identify *Tuber* species morphologically (Agerer 1987–2012, Benucci et al. 2012; Gilkey 1920; Rauscher et al. 1995, Trappe et al. 1996). For each tree sampled, roots were sorted based on color and shape of the mycorrhizal association, so there were 1–3 root samples per tree that were analyzed. Representative root tips (approx. 10–30 mg) were placed in a 2-ml microcentrifuge tube with a 3-mm glass bead and frozen at − 20 °C until processed.

DNA was extracted using the Omega Biotek Plant DNA DS Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) and DNA was stored at − 20 °C for less than 6 weeks until processed. Quantitative polymerase chain reaction (qPCR) was used to detect DNA of *T. melanosporum* on roots as described by Parladé et al. (2013). This protocol was selected because it is

Table 1 Orchard, orchard age, county, tree density, soil type and pH, irrigation type, and weed management of the orchards surveyed for mycorrhized root tips with *T. melanosporum* and/or other ectomycorrhizal fungi

Orchard	Age (years since planting)	County	Orchard density (trees/ha)*	Brule presence	Soil type	Soil pH	Irrigation	Weed management
A	2–5	Stokes	1123	Yes	Sandy clay loam	7.2–7.4	Drip	No mulch
B	9	Surrey	444	No	Sandy clay loam	Not reported	None	No mulch; manual weeding
C	15	Stokes	N/A	Yes	Sandy clay loam	7.7	Drip	No mulch; manual weeding
D	8–10	Yadkin	990	No	Sandy clay loam	< 7	None	No mulch; manual weeding
E	5	Durham	1135	No	Silt loam	Not reported	Drip	Plastic mulch
F	6	Durham	N/A	No	Silt loam	Not reported	Drip	Plastic mulch
G	5	Haywood	370	Yes	Clay loam	7.4	Drip	No mulch; manual weeding

*N/A = Orchard density was not provided by the orchard owner

more sensitive than traditional PCR methods. Samples were prepared for qPCR using the KAPA Probe Force qPCR Master Mix Universal (MilliporeSigma, St. Louis, MO, USA) at the following concentrations: each primer at 800 nM, probe at 200 nM, 5 μl of DNA template, and nuclease-free water for a final amount of 20 μl in a 0.2-μl tube. Cycling conditions were as follows: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. Positive controls of dried gleba from an ascocarp of *T. melanosporum* (8 mg to 8 ng) were included with each assay. Non-colonized root tips from *Quercus robur* and *Corylus avellana* were assayed in the same reaction to ensure the primers did not amplify root DNA. To ensure DNA extractions were successful, DNA from the cytochrome oxidase region of plant tissue was amplified with primers COX-F and COX-R and visualized by the probe COX-P labeled with the 5′-terminal FAM reporter dye, ZEN internal quencher, and the 3′-terminal Iowa Black FQ quencher. Reaction conditions and reagent concentrations were as described in Weller et al. (2000).

A tree was considered colonized by *T. melanosporum* if any root sample from that tree tested positive for *T. melanosporum*. For root samples where *T. melanosporum* was not detected or where contaminating mycorrhiza was observed microscopically, a partial region of the internal transcribed spacer (ITS1 and ITS2) was amplified from the extracted DNA using ITS1 and ITS4 (White et al. 1990) and sequenced using Sanger sequencing (MCLAB, South San Francisco, CA) in an attempt to identify the mycorrhizal fungus present. The resulting sequences were edited and aligned using the Geneious® 10.1.2 (Biomatters, Ltd., Auckland, NZ) and compared to accessions in GenBank.

Results and discussion

Tuber melanosporum was detected in all seven orchards sampled and from the root tips of 82 trees ($n = 95$) (Table 2). In six of seven orchards, *T. melanosporum* was detected on the roots of every tree; however, in Orchard E, *T. melanosporum* was only detected on the roots from two of fifteen trees sampled (Table 2). Positive detections of *T. melanosporum* from mycorrhizal trees of *Quercus* spp. and *Corylus avellana* 10 to 15 years after planting suggest that these orchards have the potential to produce European black winter truffles in NC, but the presence of the fungal mycorrhizal association does not guarantee production of the ascocarp and other factors may be involved in the lack of truffle production. Rubini et al. (2011) confirmed the heterothallic nature of the fungus where two mating types, MAT1-1 and MAT1-2, must both be present and in close contact for sexual reproduction, and therefore truffle formation, to occur. Further, recent studies suggest that in addition to the mating types, there are two genotypes that also must be in close contact for sexual reproduction (De la Varga et al. 2017). Soil characteristics may also be at play such as soil pH, soil texture, irrigation regimes, tree health, and the competition presented by native ectomycorrhizae (Lefevre 2012; Rubini et al. 2012). But the relationship between root colonization and ascocarp production is not well understood in North America and needs further research.

Tuber melanosporum was detected in at least one tree species of all seven tree species tested in this study (*Quercus robur*, *Q. pubescens*, *Q. ilex*, *Q. cerris*, *Q. ilificolia*, *Q.* sp., *Corylus avellana*) (Table 2). These results are consistent with previously published reports of the host range and cultivation of the European black winter truffle (Gryndler 2016).

This study represents the most comprehensive survey on the status of the European black winter truffle fungus in orchards in NC. Bonito et al. (2011) examined orchards across Oregon, Tennessee, and North Carolina and identified *T. melanosporum* and an undescribed species of *Tuber* in NC, but less than three orchards were surveyed in each state. Berch and Bonito (2014) reported similar results from truffieres in British Columbia, Canada, and found *T. melanosporum* and *T. aestivum* persisting where expected, similar to the results from our study. Other species of *Tuber*

Table 2 Tree species of the black Périgord truffle orchards sampled, the frequency of occurrence of *Tuber melanosporum* and other ectomycorrhizal fungus detected by qPCR (*T. melanosporum* only) and DNA sequencing

Orchard	Tree		Trees sampled ($n = 95$)	Trees colonized by *T. melanosporum* (no.)	Trees colonized by other fungi (no.)	Other fungi detected	GenBank accession match	% identity	Sequence length (bp)
	Common name	Latin name							
A $n = 15$	English oak	*Quercus robur*	8	8	1	*Scleroderma areolatum*	KT695390	99.6	704
	Common hazel	*C. avellana*	6	6	1	Sordariales	JN802315	93.3	409
	Downy oak	*Q. pubescens*	1	1	1	*T. lyonii*	EU268567	97.7	500
					1	Uncultured Sclerodermataceae	AJ879652	98.1	609
B $n = 10$	Common hazel	*C. avellana*	10	10	1	*Tarzetta catinus*	FM206478	99.6	594
					1	Uncultured Thelephorales	KF000687	98.0	634
C n = 9	Holly oak	*Q. ilex*	1	1					
	Common hazel	*C. avellana*	6	6					
	Oak	*Quercus* sp.	1	1					
	Turkey oak	*Q. cerris*	1	1					
D $n = 16$	Scrub oak	*Q. ilicifolia*	2	2					
	Common hazel	*C. avellana*	10	10					
	English oak	*Q. robur*	4	4					
E n = 15	English oak	*Q. robur*	2	1	1	Basidiomycota*			
	Common hazel	*C. avellana*	13	1	6	Basidiomycota*			
					1	*T. lyonii*	FJ748911	99.7	580
					1	Uncultured *Tomentella* sp.	FJ210775	96.2	587
F $n = 18$	Common hazel	*C. avellana*	16	16	3	*T. canaliculatum*	GQ221456	98.2	437
							GQ221456	98.2	438
							GQ221456	98.1	469
					2	Uncultured ectomycorrhiza (Pezizaceae)	FJ197013	96.7	569
					1	*T. brennemanii*	KU186932	100	509
	Holly oak	*Q. ilex*	1						
	English oak	*Q. robur*	1						
G $n = 12$	Common hazel	*C. avellana*	12	12	1	*S. bovista*	AB099901	96.0	605

*Determined by morphology and presence of clamp connections

(*T. anniae*, *T. beyerlei*, *T. borchii*, *T. brumale*, and *T. menseri* nom. Prov.) also were present, but were not expected. To our knowledge, there are no other published reports on the occurrence of *T. melanosporum* in established truffle orchards from North America.

Although *T. melanosporum* was detected on 86% of the trees sampled, we also detected other ectomycorrhizal fungi in all seven orchards tested based on microscopic examination (data not reported) and DNA sequence analysis (Table 2). Of the 95 trees sampled, roots were separated into a total of 126 root samples and 98 of these tested positive for *T. melanosporum*. Of the 28 root samples that tested negative for *T. melanosporum*, DNA from 14 root samples was successfully sequenced. An insufficient number of root tips or presence of more than one fungus was likely the cause of the failed sequencing root samples. Based on sequences, *Scleroderma bovista* Fr. was detected at Orchard G and *S. areolatum* Ehrenb. and an unknown species in the family Sclerodermataceae were detected in Orchard A. In both cases, these fungi co-occurred on the roots from the same tree(s) where *T. melanosporum* and other species of *Tuber* also were detected (Table 2). *Scleroderma* species are known to persist in truffle orchards and nurseries (Hall et al. 2007), although it is unknown if the *Scleroderma* species detected in this study were introduced on the inoculated trees or were naturally present in soil or on the roots of other plant hosts in the orchard. Regardless, it has been suggested that the presence of *Scleroderma* species is indicative of a productive *T. melanosporum* orchard (Morcillo et al. 2016; Hyson 2013), but this relationship is unknown and was not evaluated in this study. Since *Scleroderma* species are native to NC and their optimum soil pH is similar to that of *T. melanosporum* (Hall et al. 2007), it is not surprising that they were detected in the same soils.

Tuber lyonii Butters, a truffle native to the eastern United States (Trappe et al. 1996), was detected in Orchards A and C on *Q. pubescens* and *C. avellana*, respectively. This truffle has become more commonly known as the pecan or Texas truffle and is harvested in sufficient quantities to be commercially available in Georgia (Smith et al. 2012), NC, and possibly other states. In its natural environment, associated hosts include species of *Carya*, *Corylus*, and *Quercus* (Ge et al. 2017, Hall et al. 2007, Trappe et al. 1996). Hall et al. (2007) previously detected this truffle colonizing roots from adjacent colonized forest trees near the orchard. We did not sample outside any of the orchards so it is unclear where the fungus identified in this study originated. Regardless, detecting contaminating species of native or non-native *Tuber* is not uncommon in truffle orchards (Berch and Bonito 2014; Linde and Selmes 2012).

Tuber canaliculatum Gilkey, first described in California in 1920 (Gilkey), also is native to the eastern United States (Lefevre 2012) and was detected on three trees in Orchard F (Table 2). Similar to *T. lyonii*, it has a broad host range including oak (*Quercus*) and pine (*Pinus*) (Benucci et al. 2012) and has been reported in multiple states in the eastern United States. Its role, however, in truffle orchards is not well documented. Regardless, it is possible the orchard trees were colonized by hyphae from host trees in the environment given its widespread occurrence.

Tuber brennemanii A. Grupe, Healty, and M.E. Sm., a newly described species (Grupe et al. 2018), was detected on *C. avellana* in Orchard F. Recently, this truffle has been reported as common in pecan orchards (Grupe et al. 2018) and appears to be widespread, and possibly native, in the eastern United States (Bonito et al. 2010; Healy et al. 2016). The widespread occurrence of native truffle species may present a challenge to successful production of the European black winter truffle in North Carolina.

Other fungal groups known to include ectomycorrhizal fungi were detected in Orchards A (uncultured Sordariales), B (*Tarzetta catinus* and uncultured Thelephorales), E (uncultured *Tomentella* sp.), and F (Pezizaceae). Sequences for these detections were only able to confirm to order, family, or genus. As with *Scleroderma*, these ascomycetous fungi are common mycorrhizal fungi in truffle orchards (Hall et al. 2007), and have been reported elsewhere as co-occurring with *T. melanosporum* on host trees (Taschen et al. 2015). The role of these fungi in the orchards is not well understood, but more studies are beginning to investigate the dynamics of ectomycorrhizae in truffle orchards (Guerin-Laguette et al. 2013; Taschen et al. 2015).

There were no ascocarps found during the sampling period of this study; however, ascocarps were detected in some orchards after sampling occurred. Four truffles were found in Orchard G one year after these samples had been processed in 2017, two were found in 2018, and over 40 were harvested during the 2019–2020 season. Of the six truffles harvested in 2018 and 2019, five of these truffles were confirmed to be *T. melanosporum* based on qPCR and DNA sequence analysis. One truffle was confirmed to be the Burgundy truffle (*Tuber aestivum* Vittad. [=*T. uncinatum*]) based on ascospore morphology and DNA sequence analysis. Given that this truffle is not native to North America, this suggests that the inoculum used for colonization of trees represented a mixture, but this cannot be confirmed. Another orchard in NC that was not included in this study and remains anonymous reported at least two ascocarps of *T. brumale* found in 2018 based on ascospore morphology and DNA sequence analysis (unpublished data). This is the first report of *T. aestivum* and *T. brumale* as contaminants in European black winter truffle orchards in NC, although mycorrhizae of *T. aestivum* and *T. brumale* were not detected on any of the root tips in the samples we assayed in this study. It is not unusual to find other *Tuber* species in orchards or for non-target truffles to be introduced outside their native range (Bonito et al. 2010; Guerin-

Laguette et al. 2013). In North America, Bonito et al. (2011) found *T. indicum*, the Chinese truffle, in a forest setting in Oregon. Although we did not find *T. indicum* in this study, this truffle is the most feared as it is morphologically similar to *T. melanosporum* and yields a lower market price. Berch and Bonito (2014) identified *T. aestivum* in *T. melanosporum* truffle orchards in British Columbia, Canada. Typically, tree seedlings are inoculated with ascospores from truffle ascocarps and the spores germinate to colonize the developing root tips. The sporocarp of *T. brumale* is morphologically similar to *T. melanosporum* (Morcillo et al. 2015) and could be easily mistaken and incorporated into inoculum of *T. melanosporum*. In contrast, *T. aestivum* is morphologically distinct from *T. melanosporum* (Morcillo et al. 2015) and could be easily removed before inoculation occurs.

Truffle production at commercial levels in North America has been challenging (Chen et al. 2016; Berch and Bonito 2014) and factors influencing truffle formation are not well understood (Hall et al. 2007). After the sampling occurred in 2015–2016, the hazelnut trees in Orchard B were defoliated and killed by Eastern Filbert Blight ([EFB], *Anisogramma anomala*) and Orchard G has reported infection by EFB, although it is being managed with heavy pruning and preventative fungicide sprays. Establishing hazelnut trees for truffle production in eastern North America either requires planting resistant hybrid hazelnut trees or regular maintenance of a combination of fungicide sprays and pruning infected tissue, although the effect of fungicide applications to the host trees on truffle production is not known. The fungicides being recommended are preventative, as opposed to systemic, and do not move through soil well, so it is unlikely that there is much effect on truffle production. Regardless, understanding the effect of fungicide use on ectomycorrhizal host trees needs more investigation.

Based on this study, growers are strongly encouraged to test inoculated seedlings for colonization and purity of the truffle fungus of interest. At the time of this study, this was not a standard practice as the industry was emerging and practices were not well established. As a result of this study, however, the lead author on this publication has been providing a testing service for seedling and orchard producers since 2017 and has served over 40 growers during this process suggesting a shift in practice. Growers also are recommended to manage orchards according to the known practices that encourage truffle formation including maintaining proper soil pH, applying irrigation during dry summers, pruning, scouting for diseases and insects, applying fungicides and insecticides as needed, maintaining appropriate tree nutrition, managing weeds, and other factors as described in Chevalier and Sourzat (2012), Hall et al. (2007) and Morcillo et al. (2015). Additionally, it is recommended to regularly test representative trees for the presence of the target truffle fungus to help facilitate management decisions.

Although the focus of this manuscript was to document the status of the European black winter truffle in North Carolina, this study highlights the fact that the cultivation of this truffle in North America, in general, is still emerging and there is a lack of evidence-based knowledge on successful production. More research needs to be conducted to provide growers better recommendations to make this industry successful in NC and North America.

Code availability Not applicable.

Code availability Not applicable.

Authors' contributions Jeanine Davis procured the funding. Kelly Gaskill collected and processed the samples including extracting DNA. Leonora Stefanile helped in maintaining the research orchard. Inga Meadows and Suzette Sharpe also conducted DNA extractions and all of the molecular analyses. Inga Meadows prepared the manuscript. All authors contributed to the writing of the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Agerer R (1987-2012) Colour atlas of ectomycorrhizae. Einhorn-Verlag, Munich

Benucci GMN, Bonito G, Falini LB, Bencivenga M, Donnini D (2012) Truffles, timber, food, and fuel: sustainable approaches for multicropping truffles and economically important plants. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, current knowledge and future prospects, soil biology series, vol 34. Springer, Berlin Heidelberg, pp 163–189

Berch SM, Bonito G (2014) Cultivation of Mediterranean species of Tuber (Tuberaceae) in British Columbia, Canada. Mycorrhiza 24: 473–479

Bonito G, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19:4994–5008

Bonito G, Trappe JM, Donovan S, Vilgalys R (2011) The Asian black truffle *Tuber indicum* can form ectomycorrhizas with North American hosts plants and complete its life cycle in non-native soils. Fungal Ecol 4:83–93

Boutekrapt A, Chevalier G, Jc P, Dexheimer J (1990) Mycorrhization par *Tuber melanosporum* Vitt de vitroplants de *Quercus robur* L et *Quercus pubescens* Willd. Agronomie 10:127–132

Chen JJ, Murat C, Oviatt P, Wang Y, Tacon FL (2016) The black truffles *Tuber melanosporum* and *Tuber indicum*. In: Zambonelli A, Iotti M, Murat C (eds) True truffles (Tuber spp.) in the World: Soil Ecology, Systematics, and Biochemistry, Soil Biology series, vol 47. Springer, Berlin Heidelberg, pp 19–32

Chevalier G, Grente J (1979) Application pratique de la synthèse ectomycorhizienne: production à grande échelle de plants mycorhizés par la truffe (*Tuber melanosporum* Vitt.). Mushroom Sci 10:483–505

Chevalier G, Sourzat P (2012) Soils and techniques for cultivating *Tuber melanosporum* and *Tuber aestivum* in Europe. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, current knowledge and future prospects, soil biology series, vol 34. Springer, Berlin Heidelberg, pp 163–189

De la Varga H, Tacon FL, Lagoguet M, Todesco F, Varga T, Miquel I, Barry-Etienne D, Robin C, Halkett F, Martin F, Murat C (2017) Five years investigation of female and male genotypes in Périgord black truffle (*Tuber melanosporum* Vittad.) revealed contrasted reproduction strategies. Environ Microbiol. https://doi.org/10.1111/1462-2920.13735

Ge Z-W, Brenneman T, Bonito G, Smith ME (2017) Soil pH and mineral nutrients strongly influence truffles and other ectomycorrhizal fungi associated with commercial pecans (*Carya illinoiensis*). Plant Soil 418:493–505

Gilkey H (1920) Two new truffles. Mycologia 12:99–101

Grupe AC, Sulzbacher MA, Grebenc T, Healy R, Bonito G, Smith M (2018) *Tuber brennemanii* and *Tuber floridanum*: two new *Tuber* species are among the most commonly detected ectomycorrhizal taxa within commercial pecan (*Carya illinoiensis*) orchards. Mycologia 110(4):780–790

Gryndler M (2016) True truffle host diversity. In: Zambonelli A, Iotti M, Murat C (eds) True truffles (tuber spp.) in the world: soil ecology, systematics, and biochemistry, soil biology series, vol 47. Springer, Berlin Heidelberg, pp 267–281

Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y (2013) Mycorrhiza analyses in New Zealand truffières reveal frequent but variable persistence of *Tuber melanosporum* in co-existence with other truffle species. Mycorrhiza 23:87–98

Hall IR, Brown GT, Zambonelli A (2007) Taming the truffle: the history, Lore, and science of the ultimate mushroom. Timber Press, Inc., Portland

Healy RA, Zurrier H, Bonito G, Smith M, Pfister DH (2016) Mycorrhizal detection of native and non-native truffles in a historic arboretum and the discovery of a new north American species, *Tuber arnoldianum* sp. nov. Mycorrhiza 26:781–792

Hyson M (2013) Truffles: their underground story: the dragon's nest photo album: a growers guide to underground structures related to growing truffles. CreateSpace Independent Publishing Platform, Charleston

Lefevre C (2012) Native and cultivated truffles of North America. In: Zambonelli A, Bonito G (eds) Edible ectomycorrhizal mushrooms. Soil biology series, vol 34. Springer, Berlin Heidelberg, pp 209–226

Linde CC, Selmes H (2012) Genetic diversity and mating type distribution of *Tuber melanosporum* and their significance to truffle cultivation in artificially planted truffières in Australia. Appl. Environ Microbiol 78:6534–6539

Michaels T (1982) *In vitro* culture and growth modeling of *Tuber* spp. and inoculation of hardwoods with *T. melanosporum* ascospores. Dissertation, Oregon State University

Morcillo M, Sanchez M, Vilanova X (2015) Truffle farming today. Micologia Forestal & Aplicada, Barcelona

Morcillo M, Parladé J, Pera J, Sanchez M, Vilanova X (2016) Evolution of *Tuber melanosporum* mycelium concentration in the soil throughout the year and evaluation of fungal biodiversity in productive and non-productive trees. In: Eighth International Workshop on Edible Mycorrhizal Mushrooms. Cahors, France. p 109. https://trufflefarming.wordpress.com/2017/01/27/new-results-from-our-research-with-spanish-wells-with-black-truffle-spores/. Accessed 25 Mar 2020

North American Truffle Growers Association (2020a) About. https://trufflegrowers.com/about/. Accessed 03/25/2020

North American Truffle Growers Association (2020b) Truffles. https://trufflegrowers.com/truffles/. Accessed 03/25/2020

O'Neill M (2007) Coveted, French, and Now in Tennessee. New York Times. https://www.nytimes.com/2007/02/28/dining/28truf.html#:~:text=CHUCKEY%2C%20Tenn.,and%20the%20North%20Carolina%20border. Accessed 05 Aug 2020

Palenzona M (1969) Mycorrhizal synthesis between *Tuber aestivum* Vitt, *Tuber brumale* Vitt, *Tuber melanosporum* Vitt, and seedlings of *Corylus avellana*. Allionia 15:121–131

Parladé J, De la Varga H, De Miguel AM, Sáez R, Pera J (2013) Quantification of extra radical mycelium of *Tuber melanosporum* in soils from truffle orchards in northern Spain. Mycorrhiza 23:99–106

Pinkas Y, Maimon M, Shabi E, Elisha S, Shmulewich T, Freeman S (2000) Inoculation, isolation and identification of *Tuber melanosporum* from old and new oak hosts in Israel. Myc Res 104:472–477

Rahma Z (2013) Mycorrhization en conditions contrôlées du chêne vert (*Quercus ilex* L.) par la truffe noire (*Tuber melanosporum* Vitt.). Dissertation, University of Oran

Rauscher T, Agerer R, Chevalier G (1995) Ektomykorrhizen von *Tuber melanosporum*, *Tuber mesentericum* und *Tuber rufum* (Tuberales) an *Corylus avellana*. Nova Hedwigia 61(3–4):281–322

Reyna S, Garcia-Barreda S (2014) Black truffle cultivation: a global reality. For Syst 23:317–328

Rubini A, Belfiori B, Riccioni C, Tisserant E, Arcioni S, Martin F, Paolocci F (2011) Isolation and characterization of *MAT* genes in the symbiotic ascomycete *Tuber melanosporum*. New Phytol 189: 710–722

Rubini A, Belfiori B, Riccioni C, Paolocci F (2012) Genomics of *Tuber melanosporum*: new knowledge concerning reproductive biology, symbiosis, and aroma production. In: Zambonelli A, Bonito GM (eds) Edible ectomycorrhizal mushrooms, current knowledge and future prospects, soil biology series, vol 34. Springer, Berlin Heidelberg, pp 57–72

Smith ME, Bonito G, Sharma J, Long J, Davis-Long B, and Brenneman T (2012) Pecan truffles (*Tuber lyonii*) what we know and what we need to know, Georgia Pecan Magazine, Spring 2012, pp. 52–58

Taschen E, Sauve M, Taudiere A, Parlade J, Selosse M-A, Richard F (2015) Whose truffle is this? Distribution patterns of ectomycorrhizal fungal diversity in *Tuber melanosporum* brûlés developed in multi-host Mediterranean plant communities. Environ Microbiol 17:2747–2761. https://doi.org/10.1111/1462-2920.12741

Trappe JM, Jumpponen AM, Cázares E (1996) NATS truffle and truffle-like fungi 5: *Tuber lyonii* (=*T. texense*), with a key to the spiny-spored *Tuber* species groups. Mycotaxon 60:365–372

Web Soil Survey (2018) Natural Resources Conservation Service, United States Department of Agriculture. https://websoilsurvey.sc.egov.usda.gov. Accessed 16 Mar 2018

Weller SA, Elphinston JG, Smith NC, Boonham N, Stead DE (2000) Detection of Ralstonia solanacearum strains with quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. Appl Environ Microbiol 66:2853–2858

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DG, Sninsky JJ, White TJ (eds) PCR protocol: a guide to methods and applications. Academic Press, New York, pp 315–322