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SHORT NOTE

Tree age influences on the development of edible ectomycorrhizal fungi sporocarps in *Pinus sylvestris* stands

Pedro Ortega-Martínez · Beatriz Águeda · Luz Marina Fernández-Toirán · Fernando Martínez-Peña

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Abstract The study of factors influencing the production and development of wild edible mushroom sporocarps is extremely important in the characterization of the fungi life cycle. The main objective of this work is to determine how tree age influences the speed of sporocarp growth of edible ectomycorrhizal fungi *Boletus edulis* and *Lactarius deliciosus* in a *Pinus sylvestris* stand. This study is based on information recorded on a weekly basis every autumn between 1995 and 2008 in a set of permanent plots in Spain. Sporocarps are collected weekly, and as a result, specimens may not have reached their maximum size. The study area is a monospecific *P. sylvestris* stand. Three age classes were considered: under 30 years, between 31 and 70 years, and over 70 years. Sporocarps of *B. edulis* and *L. deliciosus* grow faster in the first age class stands than in the other two, and in the second age class stands, sporocarps are more than 50% smaller. The average weight of the picked *B. edulis* sporocarps clearly varies in the three age classes considered, with its maximum in the first age class (127 g and 6.8 cm cap diameter), minimum in the second age class (68 g and 4.7 cm cap diameter), and showing a relative maximum in the third (79 g and 4.3 cm cap diameter). *L. deliciosus* sporocarps are on average larger in the first age class (48 g and 7.4 cm cap diameter), decreasing in the second (20 g and 5.8 cm cap diameter) and also in the third (21 g and 5.3 cm cap diameter). The results show the influence of tree age in speed of sporocarp growth for the two ectomycorrhizal species.

Keywords *Boletus edulis* · *Lactarius deliciosus* · Stand age class · Scots pine

P. Ortega-Martínez · B. Águeda (✉) · L. M. Fernández-Toirán · F. Martínez-Peña
Departamento de Investigación y Experiencias Forestales de Valonsadero, Consejería de Medio Ambiente, Junta de Castilla y León, Apdo. de correos 175, 42080 Soria, Spain
e-mail: aguherbe@jcyl.es

L. M. Fernández-Toirán
Escuela Universitaria de Ingenierías Agrarias, Universidad de Valladolid, Campus "Duques de Soria", 42005 Soria, Spain

Introduction

Edible mycorrhizal mushrooms are not only a gourmet food but also a source of income for collectors (Wang and Hall 2004). In Europe, *Boletus edulis* Bull. and *Lactarius deliciosus* Fr. are considered to be two of the most highly valued edible mycorrhizal mushrooms.

Total annual worldwide consumption of *B. edulis* complex reaches between 20,000 and 100,000 tons (Hall et al. 1998). Important markets include North America, France, Italy, and Germany (Hall et al. 1998). The estimated annual production of *B. edulis* species complex from the autonomous community of Castilla y León (north Spain) adds up to 8,500 tons, being worth approximately 38 million euros (Martínez-Peña et al. 2006–2009).

Even though it is not as widely appreciated as porcini, *L. deliciosus* has consolidated markets in Europe, Asia, and North Africa, where a large amount of those sporocarps are commercialized and comprise a significant source of income (Boa 2004).

Seedlings mycorrhizated with *L. deliciosus* are available in nurseries throughout the world, and the scientific community is making an effort to facilitate the production of seedlings mycorrhizated with *B. edulis* (Águeda et al. 2008b; Ceruti et al. 1987; Duñabeitia et al. 1996; Olivier et

al. 1997). Nowadays, both species have to be collected from the wild with variable and unpredictable harvesting from year to year (Cannon and Kirk 2007; Murat et al. 2008).

However, factors influencing sporocarp formation are not yet apparent. Sporocarp formation is probably the most complicated stage in the life cycle of fungi. This situation is even more complex for ectomycorrhizal fungi, which require symbiotic association with a host plant. Fungal and host genes, environmental and physiological conditions, and nutritional state of mycelium and the host trigger sporocarp formation in ectomycorrhizal fungi, but the process is not fully understood to date (Murat et al. 2008).

Host development entails changes in its physiological state, which influence relations with the host's associated fungal ectomycorrhizal community and, consequently, its fructification.

The aim of this study is to establish a relationship between stand age class and the growth of *B. edulis* and *L. deliciosus* sporocarps.

Materials and methods

Study site

The study site is located in a homogeneous *Pinus sylvestris* L. stand known as "Pinar Grande." It is a 12,533-ha area situated in the Sistema Ibérico mountain range, in the inner north-east zone of the Iberian Peninsula. Altitude varies between 1,097 and 1,543 m a.s.l. with dominating west and east orientations.

Soils are acidic brown-earths or illuvial, with marked acid pH (4–5), sandy loam to sandy texture, low holding capacity, and low fertility.

Medium annual rainfall is 865 mm/year, 69 mm/year falling in July and August and 132 mm/year in September and October. Medium annual temperature is 8.8°C, with July being the warmest month (17.4°C). The frost period begins in November and ends in April, with frequent frosts in late spring and early autumn. Scots pines are felled every 100 years. The forests are silviculturally managed by clear cutting with soil movement and sowing.

In this area, 119 species of epigeous macromycetes fungi have been recorded. They belong to 51 genera and more than half are members of *Russula* Pers., *Cortinarius* (Pers.) Gray, *Tricholoma* (Fr.) Staude, *Amanita* Pers., *Lactarius* Pers., *Collybia* (Fr.) Staude, *Cystoderma* Fayod, *Hebeloma* (Fr.) P. Kumm., *Mycena* (Pers.) Roussel, and *Suillus* Gray (Martínez-Peña 2009).

B. edulis and *L. deliciosus* average autumnal sporocarp production in Pinar Grande between 1995 and 2008 by stand age class is detailed in Table 1 (Martínez-Peña 2009).

Sampling design

A random sampling design was performed by stand age, according to the forest management plan: 0–15, 16–30, 31–50, 51–70, 71–90, and over 90 years old. Three plots per age class were installed, providing a total of 18 sampling plots.

Each sampling plot covers a rectangular-shaped area of 150 m^2 (measuring 35×5 m). Its size and form were established in line with previous studies by Fernández-Toirán (1994), Kalamees and Silver (1988), and Ohenoja (1989) that use rectangular plots with a minimum area of 100 m^2. Plots were fenced to prevent harvesting and trampling and were at least 500 m from stands corresponding to another age class.

For the present study, plots were regrouped by age in three categories: under 30 years, between 31 and 70 years, and over 71 years, which represent three clearly different stages in the life of a stand: young, mature, and old.

Sampling was performed on a weekly basis from week 35 to 50 every year from 1995 to 2008, as this period corresponds with most sporocarp emergence. All fully developed sporocarps were collected and identified to species level in the laboratory.

Biometric study focused on two edible ectomycorrhizal fungal species: *B. edulis* and *L. deliciosus*. Speed of sporocarp growth is the analyzed dependant variable. Fresh weight (g) and cap diameter (cm) of each sporocarp collected was recorded for both species. Due to the fact that sporocarps are collected every week, the specimens may have not reached their maximum size.

Data analyses

Statistical analyses were performed using STATGRAPHICS Plus 5.1 (Statistical Graphics Corp., Warrenton, VA, USA).

The effect of age stand in the speed of sporocarp growth for *B. edulis* and *L. deliciosus* was analyzed by the weight that sporocarps reach in 1 week using ANOVA. As data proved to

Table 1 *Boletus edulis* and *Lactarius deliciosus* autumnal sporocarps mean fresh weight production (kg/ha) between 1995 and 2008 in Pinar Grande by stand age class (extracted from Martínez-Peña 2009)

	Pinus sylvestris age class stand (years)			Mean
	Under 30	31–70	Over 71	
Boletus edulis	16.2	84.9	21.9	30.3
Lactarius deliciosus	17.9	3.1	14.9	9.1

be unbalanced, the general linear model procedure was applied. Tukey's multiple rank test was used for the multiple mean comparison (Einot and Gabriel 1975). Data were subjected to a logarithmic transformation for normalization purposes.

Speed of sporocarp growth was also analyzed from the cap diameter that sporocarps reach in 1 week using the Kruskal–Wallis test for comparing medians due to the fact that normal distribution and homogeneity hypothesis were not reached although logarithmic transformation was performed.

Results

B. edulis and *L. deliciosus* data analysis show conclusive results on the impact of stand age class in the speed of sporocarp growth. Standard deviation analyses provide valuable information, although the variable weight had been transformed with a logarithm function to comply with the model assumptions.

The average weight of *B. edulis* sporocarps are clearly different for the three stand age classes considered. The maximum is found in the first age class stand, 127 g, and decreases for other stands, 68 and 79 g, respectively (Table 2 and Fig. 1). *B. edulis* sporocarps that developed under the first age class stand have a larger cap diameter after 1 week (Table 2).

L. deliciosus sporocarps occurring under the first age stand are also of significant weight and are included in the major production stage of the stand (Tables 1 and 3). The maximum is found in the first age class stand, 47 g, and decreases for the other two stands, 20 and 21 g, respectively (Table 3 and Fig. 2). There are not enough data to establish any conclusions regarding cap diameter, but *L. deliciosus* developed under the first stand age class reach larger sizes in 1 week (Table 3).

B. edulis and *L. deliciosus* developed under the first age class stand are heavier than those growing in the two other studied categories. The weight of sporocarps in the second age class is nearly 50% lower than in the first.

Discussion

The variable under evaluation is sporocarp growth in 1 week, as sporocarps are picked every week and not at the end of their development. Both species grow faster when they are associated with pine trees in the first age class.

B. edulis and *L. deliciosus* sporocarps occurring in the first age class are larger than in the rest of the classes and nearly 50% heavier in the first than in the second.

This similar behavior is surprising, considering the great differences between these two species. Firstly, *B. edulis* sporocarps are usually collected in old stands (Dighton and Mason 1985), as they are representative of late succession stages in the ectomycorrhizal fungal community. Conversely, *L. deliciosus* sporocarps are usually collected in young stands, being a typical member of early succession stages. In *Pinus pinaster* Ait. stands, *L. deliciosus* reaches its maximum production in the first and in the last age classes (Ágreda and Fernández-Toirán 2008; Fernández-Toirán et al. 2006).

Another main difference between the two species is in their microscopic structures. *B. edulis* is a long-distance species (Agerer 2001), with highly differenced rhizomorphs, formed by hydrophobic hyphae (Águeda et al. 2008a). These features make *B. edulis* highly efficient in transporting water and nutrients to the host and very resistant to ecologic adversities due to its ability to absorb water from deep soil horizons (Lilleskov et al. 2009). *L. deliciosus*, however, is a medium distance subtype smooth species (Agerer 2001), with rhizomorphs formed by undifferentiated hyphae (Uhl 1988). Furthermore, the manner in which a determined species extends in the soil

Table 2 Descriptive statistics for *Boletus edulis* sporocarps weight (g) and cap diameter (cm) reached in 1 week by *Pinus sylvestris* stand age class (years)

	B. edulis sporocarps weight (g)						*B. edulis* sporocarps cap diameter (cm)		
	Under 30		31–70		Over 71		Under 30	31–70	Over 71
Frequency (number of analyzed sporocarps)	138		721		270		124	675	218
	Weight	Log (weight)	Weight	Log (weight)	Weight	Log (weight)			
Mean	126.8	2.1	68.1	1.8	78.9	1.9	6.8	4.7	5.3
Median	92.5	2.0	40.0	1.6	60.0	1.8	6.0	4.0	5.0
Standard deviation	125.9	2.1	83.5	1.9	65.1	1.8	3.8	2.3	2.5

Sporocarps were picked every week during autumn between 1995 and 2008

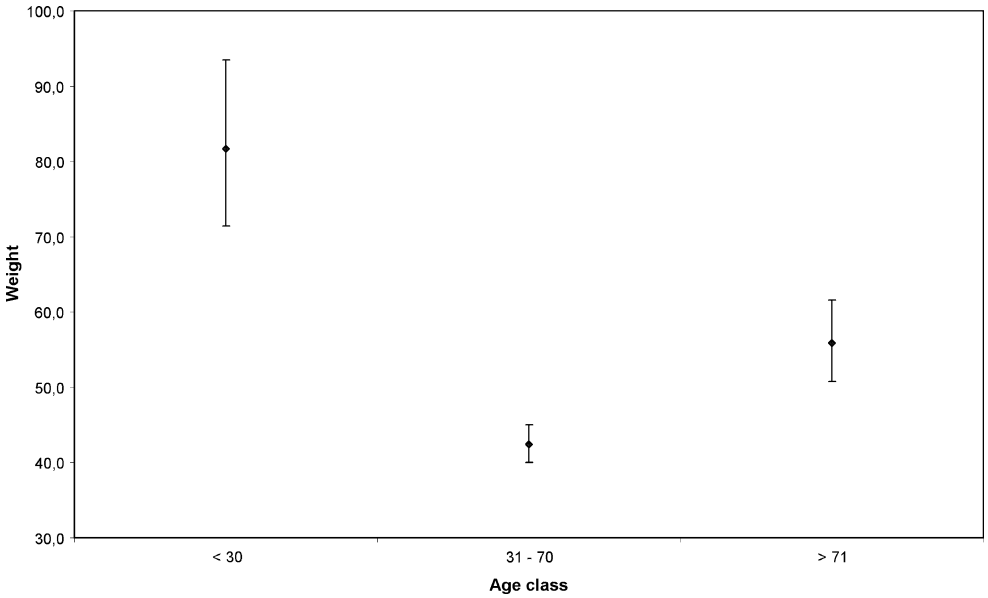

Fig. 1 Means and Tukey intervals (95%) for *Boletus edulis* medium sporocarp weight reached in 1 week by stand age class. *Pinus sylvestris* stand age classes represented in years and *B. edulis* sporocarp weight in g

and the differentiation of their extramatrical mycelium and rhizomorphs in ectomycorrhizal fungi are extremely important ecological factors for tree performance (Agerer 2001).

Extramatrical mycelium and rhizomorphs are structures that specialized in water and nutrient collection and transport to the host. Mycorrhizal fungi not only cover the portion of the root where absorption of nutrients predominates but also create an extensive and dynamic mycelial network ranging far from the root tip and even into the bedrock (Allen 2009; Lilleskov et al. 2009).

Sporocarp formation is probably the most complex phase in the life cycle of a fungus. It involves dramatic changes of the growth pattern: From a loose mesh of undifferentiated hyphae, a compact multihyphal structure is formed and devoted to sexual reproduction (Murat et al. 2008). The situation is even more complex for ectomycorrhizal fungi, as the sporocarp represents a unique step in a complex life cycle, which requires the symbiotic association with a plant host (Murat et al. 2008).

Abiotic factors, such as light, temperature, humidity, and nutrient availability, exert a decisive influence on sporocarp formation (Erland and Taylor 2002; Murat et al. 2008). These factors could be considered homogeneous in this study. In general, sporocarp development in the ectomycorrhizal fungi is induced by temperature drop, high humidity, neutral or slightly acidic soil, and solar light, although universal conditions triggering development is still unknown.

Nutrient availability is a factor of major importance in sporocarp formation. Although the composition of the ectomycorrhizal fungal community on the roots is more or less constant throughout the seasons, hyphae abundance is more dynamic and variable (Koide et al. 2007). Moreover, hyphae abundance of soil mycelia are of vital importance, although there is not always a direct relationship between mycelial volume and sporocarp production (Gardes and

Table 3 Descriptive statistics for *Lactarius deliciosus* sporocarp weight (g) and cap diameter (cm) reached in 1 week by *Pinus sylvestris* stand age class stand (years)

	L. deliciosus sporocarps weight (g)						*L. deliciosus* sporocarps cap diameter (cm)		
	Under 30		31–70		Over 71		Under 30	31–70	Over 71
Frequency (number of analyzed sporocarps)	73		36		149		35	6	48
	Weight	Log (weight)	Weight	Log (weight)	Weight	Log (weight)			
Mean	47.4	1.7	20.3	1.3	21.0	1.3	7.4	5.8	5.3
Median	38.0	1.6	21.5	1.3	17.3	1.2	7.0	6.5	5.0
Standard deviation	35.5	1.6	10.4	1.0	13.1	1.1	2.5	1.6	1.8

Sporocarps were picked every week during autumn between 1995 and 2008

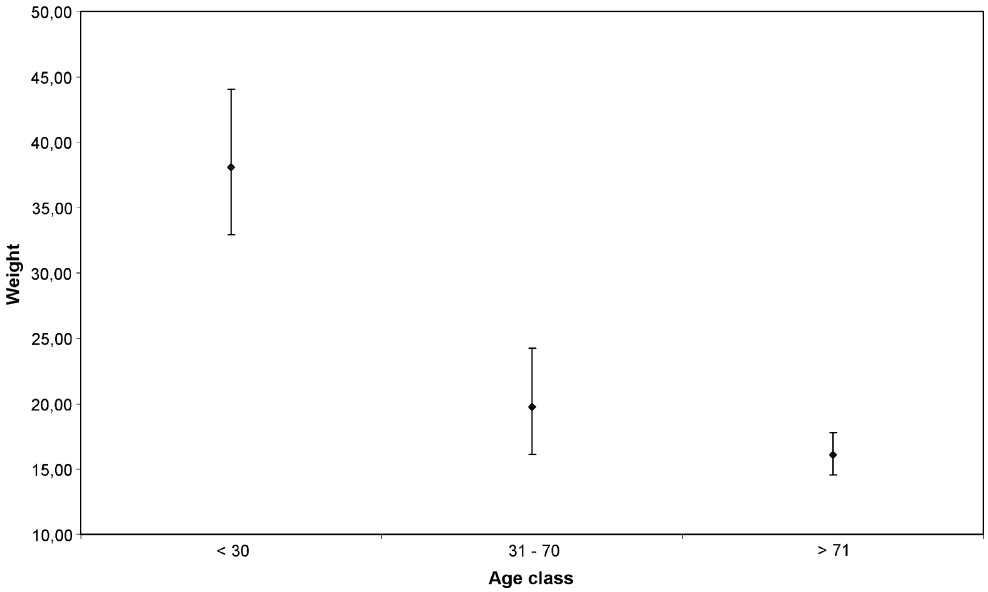

Fig. 2 Means and Tukey intervals (95%) for *Lactarius deliciosus* medium sporocarp weight reached in 1 week by stand age class. *Pinus sylvestris* stand age classes represented in years and *L. deliciosus* sporocarp weight in g

Bruns 1996) as it depends on biotic, abiotic, and physiological factors to a large extent (Erland and Taylor 2002; Smith et al. 2002). In fact, it has been demonstrated that the production of *Tuber melanosporum* Vittad. sporocarps are related to the decrease of extraradical mycelium in the soil (Suz et al. 2008).

Physiological conditions of the host are crucially important factors that trigger sporocarp formation in ectomycorrhizal fungi (Flegg and Wood 1985).

Scots pine in the *Sistema Ibérico* mountain range has its maximum current annual increment in volume when it is 40–60 years old, while maximum current annual increment in height takes place when it is 20–40 years old (Montero et al. 2008). Main root growth stops between 40 and 50 years old (Bravo and Montero 2008). Scots pine in Spain has its maximum relative increment for basal area and volume when it is approximately 10 years old, 11%, decreasing considerably from 20 years old onwards (Montero et al. 2008). Considering these facts, it could be reasoned that host photosynthetic activity is higher when its growth is at its maximum, namely at the end of the first age class for height and basal area increment and the second age class for volume increment.

Trees require more nutrients in their early development phases when growth rates are faster. The fungus could be using these circumstances to its own advantage as it is not capable of surviving without a host and can use more nutrients, complex or otherwise, in the development of its sporocarps.

Some authors found less sporocarp biomass in old-growth stands than in younger stands (Bonet et al. 2010; Dahlberg and Stenlid 1994; Smith et al. 2002). Although those studies analyzed sporocarp biomass, not speed of growth, their results could be explained in a similar way to those presented here.

Bonet et al. (2010) revealed that stand basal area is a strong predictor of sporocarp productivity. The basal area associated with maximum sporocarp productivity coincides with the peak of annual basal area increment in the studied pine stand, when the pine trees are 20–40 years old, suggesting that resources needed for tree growth and for sporocarp production come from a common pool.

Similar positive relationships between the photosynthetic rate of the host and speed of sporocarp growth could explain the facts presented here. In the present study, *L. deliciosus* shows a higher speed of sporocarp growth in young stands, when biomass production is at its highest (Table 1). *B. edulis* has a higher speed of sporocarp growth in young stands, while its greatest biomass production is not in young stands (Table 1) because the species is not representative of early succession stages.

A clear mechanistic hypothesis as to why the speed of sporocarp growth might vary according to tree age class cannot be provided. Further investigation is necessary to obtain these results. The study of the sporocarp behavior has allowed us to increase our knowledge of the ectomycorrhizal fungi life cycle and its spatial and temporal variability, providing suitable information to subsequently develop appropriate management tools for this valuable natural resource.

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