Natural production of *Tuber aestivum* in central Spain: *Pinus* spp. *versus Quercus* spp. brûles

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Abstract

Aim of study: *Tuber aestivum* is the most widespread edible truffle, with increasing commercial interest. This species can produce carpophores with conifer hosts, in contrast with the inability of *Pinus* spp. to induce fruiting in other truffle species such as *Tuber melanosporum*. Therefore the objective is to compare the characteristics and carpophore production of *T. aestivum* brûlés associated with *Pinus* spp. versus *Quercus* spp.

Area of study: We studied the natural habitats of *T. aestivum* in the Alto Tajo Nature Reserve in central Spain.

Material and methods: During 5 years, we monitored the production of carpophores and brûlé size of 145 *T. aestivum* brûlés associated with *Pinus nigra* subsp. *salzmanni* and *P. sylvestris* and *Quercus ilex* subsp. *ballota* and *Q. faginea* hosts. Statistical treatment was performed using the Statistica Program v. 6.

Main results: The size of brûlés associated with *Pinus* was significantly smaller than that of brûlés associated with *Quercus*. However, carpophore production per brûlé, and especially for brûlés of similar size, was greater when the host plant was a pine. After accounting for brûlé size, the production of brûlés associated with *Pinus* spp. was 2.23 (95% CI, between 1.35 and 3.69) and 1.61 (95% CI, between 1.02 and 2.54) times greater than the production of brûlés associated with *Quercus faginea* and *Q. ilex* subsp. *ballota*, respectively.

Research highlights: The considerable ability of *Pinus nigra* subsp. *salzmanni* and *P. sylvestris* to form effective brûlés and to produce carpophores of *Tuber aestivum* in natural conditions was clearly demonstrated, and suggest that those species can be of use in the culture of *T. aestivum*.

Key words: summer truffle; *Tuber aestivum*; truffle culture; truffle ecology; *Pinus* spp.; *Quercus* spp.

Introduction

Tuber aestivum Vittad. (summer truffle) is the most cosmopolitan edible truffle. *Tuber eastivum* has a widespread distribution from Sweden to Morocco, from UK to Ukraine in Europe, but it can also be found as far as China. However, despite increasing commercial interest, the wide ecological and geographical amplitude of *T. aestivum* has hindered the ecological knowledge of this valuable species (Montechi and Sarasini, 2000; Arredondo-Ruiz *et al*., 2014).

The production points of *T. aestivum*, known as burns or brûlés, are characterized by clearings in the vegetation, a result of the phytotoxic capacity of the *Tuber* mycelia (Plattner and Hall, 1995; Hall *et al*., 2003;

Pomarico *et al*., 2007). *Tuber aestivum* dominates the ectomycorrhizal populations within their brûlés, excluding other mycorrhizal species. There also may be a "host effect", where plants can allocate resources selectively to preferred and ideal ectomycorrhizal associates. All host species present varying morphological characteristics that condition mycorrhization under particular environment (Benucci *et al*., 2011).

Tuber aestivum can form mycorrhizal associations with more than 20 species of host plants, including species of the genus *Castanea*, *Cistus*, *Corylus*, *Fagus*, *Ostrya*, *Tilia*, *Picea*, *Carya*, *Pinus* and *Quercus* (García-Montero *et al*., 2008; Wedén *et al*., 2009; Benucci *et al*., 2012; Stobbe *et al*., 2013). However, Chevalier (2009) noted that conifers may be more efficient in producing carpophores of *Tuber aestivum* than other host plants, because conifers may have lower water requirements than hardwoods. Most of the

Chinese edible black truffles, including *T. aestivum*, *T. indicum* Cooke and Massee complex and *T. pseudoexcavatum* Wang, G. Moreno, L. G. Riousset, J. L. Manjón and G. Riousset are produced in coniferous forests (Wang *et al*., 2008).

The association between European pines and highly prized truffles is also well-known. However, there is little information estimating the productivity of edible black truffles in European pine forests. In Central Spain, García-Montero *et al*. (2007) monitored the production of 433 *Tuber melanosporum* Vittad. (black truffle) brûlés for 7 years, in mixed forests of pines and oaks. They concluded that *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco and *P. sylvestris* L. hinder the production of *Tuber melanosporum* carpophores and, therefore, pine species are not recommended for black truffle culture.

In those mixed forests of Central Spain, there was also a significant presence of *Tuber aestivum* brûlés, competing against *T. melanosporum* brûlés. García-Montero *et al*. (2008) compared the soil properties of brûlés from those two truffle species, both associated with oaks [*Quercus faginea* Lam. and *Q. ilex* L. subsp. *ballota* (Desf.) Samp.]. They concluded that active carbonate was a major factor in the fruiting and aggressiveness of *Tuber melanosporum* versus *T. aestivum*. However, no evidence of oak host species effect was revealed (single species stands of *Quercus faginea*, *Q. ilex* subsp. *ballota*, and mixed-species stands) in the production of carpophores of either *Tuber melanosporum* or *T. aestivum* (García-Montero *et al*., 2014).

Despite current interest in the harvest and culture of *Tuber aestivum,* there is little information about its ecology and, in particular, the effect of host species in the production of valuable carpophores. Thus, the objective of this study is to compare the characteristics and carpophore production of *T. aestivum* brûlés associated with *Pinus* spp. versus *Quercus* spp. in natural forests of central Spain. The results of this study could provide significant information to evaluate the suitability of *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* for *Tuber aestivum* cultivation.

Material and methods

Study area

Professional truffle hunters and the authors monitored 145 natural *Tuber aestivum* brûlés in Mediterranean forests for 5 years (2007-2012). The brûlés were located in 7 municipalities of central Spain (Sigüenza and Peralejos de las Truchas, province of Guadalajara; Medinaceli, Arcos de Jalón and Chaorna, province of Soria; Masegosa and Beteta, province of Cuenca). The host plants were *Q. ilex* subsp. *ballota*, *Q. faginea*, *P. nigra* subsp. *salzmannii* and *P. sylvestris*. The vegetation of the study area fell within the Mediterranean Ibero-Levantina Province Castellano Maestrazgo-Manchega Subprovince geobotanical classification type (Rivas-Martínez, 1987).

The study area is dominated by Jurassic and Cretaceous limestone and dolomites. Soils are lithic and rendzic leptosols with marked surface stoniness and high peaks. Elevation ranged from 1,000 to 1,450 m.a.s.l. The dominant aspect is southern. Mean annual temperature is 10.8 , and mean monthly minimum and maximum temperature is 3.3 and 18.5 , respectively. Mean annual precipitation is 650 mm (Table 1).

Data collection and analysis

Tuber aestivum has been harvested regularly in the area since the early 2000s. Carpophores are harvested with the help of trained dogs. This study was based on the collaboration with 4 truffle collectors who shared their knowledge and collection data, including the location of several harvesting points. During many field trips undertaken between 2007 and 2012 with these collectors, 145 of the collectors' preferred brûlés were selected, based on their regular production.

In each brûlé, we determined the host plant, size (m^2) of the brûlé and carpophore production. The maximum annual production of *Tuber aestivum* carpophores was calculated as the greatest quantity of fresh carpophores, in grams, picked during a harvest season, over a minimum period of 5 years. The maximum annual production (instead of the average yield) was chosen to standardize and compare the optimum yields of brûlés (in the most favorable year for each site) located in different areas (García-Montero *et al*., 2007). Harvest data were supplied by the 4 collectors who harvested those 145 brûlés regularly. The reliability of the information provided by the collectors was confirmed independently in systematic field trips made with the collectors at the time of harvesting throughout the period between 2007 and 2012. In 2012, at the end of monitoring period, we measured the longest and shortest axis and estimated brûle area

Table 1. Climatic data associated with 6 Tuber aestivum populations monitored in natural habitats in Spain (based on weather stations at Peralejos de las Truchas, Cañizares and Sigüenza)

Month	Maximum monthly temperature (°C)			Minimum monthly temperature (°C)			Average monthly temperature (°C)			Total monthly rainfall (mm)		
	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min
Jan	9.28	10.08	8.16	−2.58	−2.52	−2.70	3.09	3.68	2.25	51.16	69.46	29.40
Feb	11.01	12.30	9.74	−1.63	−1.58	−1.66	4.42	5.13	3.60	66.94	81.14	46.83
March	13.25	14.68	11.84	−0.79	−0.74	−0.90	6.25	7.05	5.45	54.11	64.70	33.27
April	16.10	17.36	15.36	2.00	2.44	1.78	9.01	9.90	8.20	104.95	116.80	96.38
May	19.58	21.45	18.14	5.04	6.13	4.50	12.39	14.13	10.90	71.30	83.02	55.25
June	23.77	25.76	21.64	7.49	8.86	6.80	15.76	17.45	13.55	47.29	55.36	33.63
July	30.64	31.72	29.08	10.30	10.90	10.00	20.62	21.13	19.80	6.13	12.16	2.00
Aug	30.81	32.10	29.12	10.66	11.30	10.34	21.02	21.63	20.05	16.77	21.00	13.52
Sept	25.32	26.82	23.35	7.33	7.34	7.30	16.26	17.23	15.20	30.89	39.05	26.44
Oct	19.81	21.34	18.16	3.08	3.13	3.06	11.58	12.50	10.73	68.92	75.32	58.63
Nov	12.56	14.26	11.42	−0.82	−0.74	−0.86	6.06	6.88	5.53	53.45	60.92	40.98
Dec	9.27	10.56	8.28	−2.78	−2.62	−3.10	3.37	4.20	2.85	75.32	99.46	30.50

using an ellipse model. In brûles with an irregular shape, we used a combination of several small ellipses. The exact geographic coordinates of these brûlés were not provided, to maintain the confidentiality of the *T. aestivum* locations.

We compared the size and *Tuber aestivum* carpophore production of the brûlés associated with different host plants species using analysis of variance. We used analysis of covariance to compare truffle production among hosts while controlling for brûlé size. The response variables were log transformed. Therefore, following Ramsey and Schaffer (1997, p: 207), we interpreted the results as multiplicative effects on the group medians. The family-wise error rate was controlled with Tukey-Kramer's procedure. All analyses were done in R v. 3.0.2 (R Core Team 2013).

Results

The average area of the 145 brûlés was 40.01 m^2 (SE, 3.66 m^2), but there was a wide range of sizes, from 1 to 314 m^2. The highest yielding brûlé produced 7.00 kg of carpophores in one season, but the mean maximum production for all 145 brûlés was 0.976 kg per brûlé and season (SE, 0.095 kg yr^{-1}). Table 2 summarizes the natural production and brûlé size data depending on the host plant of *Tuber aestivum*.

Is there a difference in truffle production among hosts?

There is convincing evidence that the maximum production of *Tuber aestivum* carpophore per brûlé

Table 2. *Tuber aestivum* brûlé maximum yield and size, by host plant

Host plant	*Quercus ilex**	*Quercus faginea**	*Pinus nigra*** and *P. sylvestris*	Total
Number of brûlés	76	47	22	145
Mean maximum yield (g y^{-1})	822.4	683.0	2,131.8	975.9
S.E.	69.9	68.7	498.2	94.9
Minimum maximum yield (g y^{-1})	100	100	100	100
Maximum maximum yield (g y^{-1})	4,000	2,000	7,000	7,000
Mean brûlé area (m^2)	35.16	56.87	20.68	40.01
S.E.	7.23	9.51	7.23	3.66
Minimum brûlé area (m^2)	6	6	1	1
Maximum brûlé area (m^2)	100	314	150	314

* *Quercus ilex* subsp. *ballota*. ** *Pinus nigra* subsp. *salzmannii*.
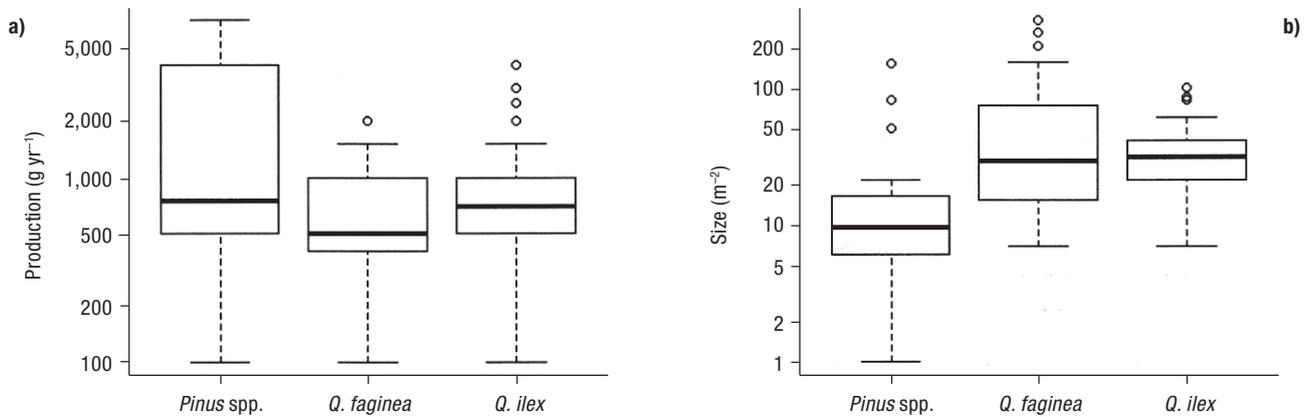
Figure 1. Boxplots of production (a) and size (b) of Tuber aestivum brûlés in central Spain, as a function of host species. The top and bottom of the box are the 25th and 75th percentiles, respectively, and the band inside the box is the median. The whiskers extend to the most extreme data point within 1.5 times the interquartile range. More extreme data points are represented by dots. Note that the ordinate is in the logarithmic scale.

differs among hosts ($F_{2,144} = 4.28$; $p = 0.016$). The estimated median production for *Pinus* spp. host is 85.4% greater than the production for *Quercus faginea* (95% CI from 12.4 to 205.9%). There is no significant difference in production between the two oak hosts, or between *Q. ilex* subsp. *ballota* and *Pinus* spp. (Fig. 1a).

Is there a difference in the size of the brûlé among hosts?

There is convincing evidence that the area of *Tuber aestivum* brûlés differs among hosts ($F_{2,144} = 14.13$; $p < 0.0001$). The estimated median brûlé area for *Quercus faginea* and *Q. ilex* subsp. *ballota* hosts is 178.8% (95% CI from 73.4 to 348.1%) and 142.1% (95% CI from 55.2 to 277.6%) greater than the area of *Pinus* spp. host, respectively. There is no significant difference in brûlé size between the two oak hosts (Fig. 1b).

Is there a difference in production among hosts, after accounting for the size of the brûlé?

Because the size of the brûlé was different among hosts, and the production was greater but the area much smaller when the host was a pine versus an oak, we compare truffle production after accounting for brûlé size. The effect of both host and brûlé size on truffle production was significant, so truffle production depends on host ($p = 0.001$) and increases with the area of the brûlé ($p = 0.001$). There was no evidence that the

relationship between production and brûlé size was different among hosts (*p*-value for the interaction, 0.13). Holding brûlé size constant, the production of *Pinus* spp. is estimated to be 123.3% (95% CI from 35.1 to 269.0%) and 60.6% (95% CI from 1.7 to 153.6%) greater than the production in *Quercus faginea* and *Q. ilex* subsp. *ballota*, respectively. There is suggestive but inconclusive evidence of a difference in production between the two oak species ($p = 0.077$) (Fig. 2).
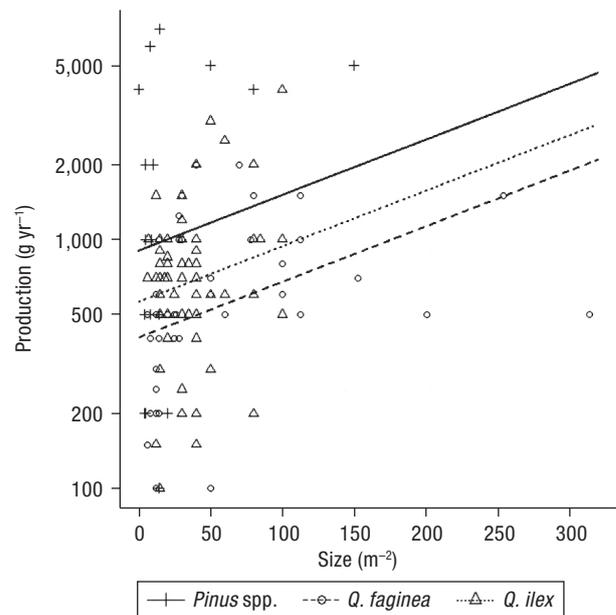

Figure 2. Relationship between *T. aestivum* production and brûlé size in central Spain. Note that the ordinate is in the logarithmic scale.

Discussion

Mediterranean pine forests are rich in mushrooms and truffles. In many regions, harvesting edible mycorrhizal species generate greater economic benefits than any other forest product (Oria, 1989, 1991). Even though species from the genus *Tuber* are one of the most valuable and sought after, and pine forests one of the most common forest types, there is very little information about truffle production in association with *Pinus* spp. (García-Montero *et al.*, 2007).

It is remarkable the significantly smaller area of the brûles associated with *Pinus* spp. versus those associated with *Quercus* spp., despite the greater production of the former (Table 2; Fig. 1). As a result, the truffle production of brûles of the same size was much greater in pines (2.23 and 1.61 times greater) that than the production in oaks. García-Montero *et al.* (2009, 2014) hypothesized that the ecological significance of the brûlé is to modify the soil environment, increasing active carbonate and exchangeable calcium and/or soil pH, to stress the host plant with nutritional deficiencies by lime induced chlorosis. The plant would respond to the stress by increasing the number of root tips and, therefore, favoring *Tuber* spp. mycorrhization. This hypothesis and our results suggest that *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* could be more sensitive to the stress caused by the *Tuber aestivum* brûlé than *Quercus* spp. Thus, to accomplish the same impact on the host plant, the fungus would have to invest fewer resources, leading to smaller brûlés and greater truffle production per unit of brûlé area.

Our results indicate that *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* may have an important role in *Tuber aestivum* cultivation because, at least in natural habitats and under certain ecological conditions, both species produced high yields of *T. aestivum* carpophores. However, new studies are necessary to evaluate whether *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* differ in their production carpophores and/or their ability to form brûles of *Tuber aestivum*, because, in the Iberian Peninsula, *Pinus sylvestris* seems indifferent to soil type while *Pinus nigra* subsp. *salzmannii* favors marl-limestone soils (Amaral Franco, 1986), as does *Tuber aestivum*. This fact suggests that *P. nigra* subsp. *salzmannii* may better tolerate increases in soil pH and/or exchangeable calcium that occurs inside *Tuber* brûlés, as proposed by García-Montero *et al.* (2009, 2014).

In summary, we found that *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* have considerable ability and facility of to form effective brûlés and to produce carpophores of *Tuber aestivum*. Although both *Pinus* are very little-known species in truffle culture, the results support their use under the appropriate climatic and soil conditions required for their growth and the development of *T. aestivum*. Moreover, we can hypothesize that other *Pinus* spp. could have a highly relevant role in the cultivation of several *Tuber* spp. In this regard, it has been observed that *Pinus nigra* subsp. *salzmannii* produces carpophores of *Tuber aestivum* (= *uncinatum*), *T. mesentericum* Vittad., *T. borchii* Vittad. and, exceptionally, *T. magnatum* Pico; *Pinus pinea* L. produces *Tuber borchii*, *T. aestivum*, *T. magnatum* and *T. indicum*; *Pinus halepensis* Mill. produces *Tuber borchii*; *Pinus tabulaeformis* Carr. var. *yunnanensis* (Franch.) Shaw produces *Tuber indicum*, *T. pseudoexcavatum* and *T. sinense* Tao and Liu; and *Pinus armandii* Franch. produces *Tuber indicum* and *T. sinense* (Pacioni 1987; Chevalier and Frochot, 1997; Granetti *et al.*, 2005; Montecchi and Sarasini, 2000; Zambonelli *et al.* 2000; Riousset *et al.*, 2001; García-Montero *et al.*, 2010). However, little is known about the actual capacity of those *Pinus* species for producing significant amounts of truffles with commercial interest.

Acknowledgements

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