

Habitats of *Tuber melanosporum* in the central Iberian Peninsula (High Tajo Basin)

Luis G. García-Montero¹, Gabriella D. Masimo², Cristina Pascual³, Miguel A. Casermeiro⁴ & José L. Manjón^{5*}

¹ Departamento de Proyectos y Planificación Rural, ETSI de Montes, Universidad Politécnica, Ciudad Universitaria s/n, 28040-Madrid, Spain

² Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Università Degli Studi di Perugia, Borgo XX Giugno 74, 06121-Perugia, Italy

³ Departamento Tecnología Química, Ambiental y de los Materiales, Universidad Rey Juan Carlos, C/Tulipán s/n, 28933-Móstoles, Madrid, Spain

⁴ Departamento de Edafología, Facultad de Farmacia, Universidad Complutense, Ciudad Universitaria s/n, 28040-Madrid, Spain

⁵ Departamento de Biología Vegetal, Facultad de Biología, Universidad de Alcalá, 28871-Alcalá de Henares, Madrid, Spain

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Abstract. In some European countries, highly prized truffles of the genus *Tuber* generate more economic benefits than any other woodland product. In this preliminary study of the ecology and truffle producing capacity of *Tuber melanosporum* in the central region of the Iberian Peninsula, we examined 433 sites producing this truffle in 8 types of high forest habitats associated with *Quercus faginea*, *Q. ilex* subsp. *ballota*, *Corylus avellana*, *Cistus laurifolius*, and *Tilia platyphyllos*. The production of this truffle in these natural, un-managed stands was confirmed in interviews conducted with 14 truffle-gatherers from the Alto Tajo Basin.

Key words: production, truffle, *Tuber melanosporum*

Introduction

Most edible forest fungi are mycorrhizal and in many Spanish mountain regions, the production of mushrooms provides higher economic returns than any other forest product. Mycorrhizal fungi form a significant part of the woodland formations of the interior Iberian Peninsula and include highly appreciated species like *Lactarius deliciosus* L. : Fr., *Boletus pinophilus* Pilát & Dermek., and *B. edulis* Bull. : Fr. along with the ectomycorrhizal hypogeous ascomycetes, or truffles, of the genus *Tuber* F.H. Wigg. (Moreno *et al.* 1986; Oria 1988, 1991; Reyna 1992).

Oria (1991) reported that habitats with calcareous soils have a lower fungus producing capacity than those with acidic

soils. The explanation for this provided by Wild (1992) and Souzart (2001) is that, although fungi tolerate a fairly wide range of pHs, their populations are reduced by competition from the abundant bacterial flora living in neutral or basic soils rich in calcium carbonate. Truffles are however an exception to this rule since calcareous, well-drained soils are the obligate habitat for most truffles. Among *Tuber* species that grow in Spain's continental calcareous high forest regions, the black Périgord truffle (*Tuber melanosporum* Vittad.) is particularly appreciated for the high market price it fetches. This truffle has accordingly been the focus of many studies by investigators in Europe, North America and even New Zealand, who have strived to evaluate its natural ecology in an effort to promote their cultivation. However, there are still evident gaps in our

*Corresponding author: e-mail: josel.manjon@uah.es

knowledge of the black truffle's biological cycle, adaptive responses to the physical environment, factors inducing fruiting, and interactions with its host plants and other fungi etc. (Callot 1999; García-Montero 2000; Montecchi & Sarasini 2000; Federation Française des Trufficulteurs 2001; Rioussset *et al.* 2001; Ricard *et al.* 2003).

Ceruti (1990) for example argued the importance of correlating ecology and physiology studies and, along with Chevalier & Dupré (1990), pointed out the need for information on optimal conditions for growth of fruiting bodies, or carpophores, and maintenance in the field of the mycorrhizae of *T. melanosporum*. Moreover, Scannerini (1992) states that efforts at incorporating biotechnology in truffle cultivation require more basic research into the biology of the mycorrhizae, mycelium and the fungus-plant relationship, as well as more research in the field over long periods. Finally, according to Chevalier (2001), cultivation of *T. melanosporum* has barely advanced and current interests in truffle production are focusing more and more on ecological approaches.

Given this need to expand current knowledge on the ecology of *Tuber melanosporum*, the present investigation was designed to characterise the habitats of this truffle in central Spain in a natural reserve denoted the Parque Natural del Alto Tajo. Studies in these areas were initiated with those of García-Montero *et al.* (1996, 2001) and García-Montero (2000), who describe the Alto Tajo as an original area for this type of study since it is a continental elevated region with its particular substrata and plants whose relationships with *T. melanosporum* are scarcely known. Our objective was to identify and describe these habitats to determine the range of environments in which *T. melanosporum* grows and thus establish a basis for future studies on the production and biology of this species in its natural environment.

Materials and Methods

Our study area included 7 wild sites of *Tuber melanosporum* production in the Alto Tajo, found within a 10 km radius between the municipal areas of Peralejos de las Truchas (Guadalajara) and Coto de Belvalle (Beteta, Cuenca). These contiguous regions have been physically described in García-Montero *et al.* (1996, 2001) and García-Montero (2000). The area is mountainous with woods of *Quercus* L. and subhumid supramediterranean pines at an altitude of over 1000 m. *T. melanosporum* has been harvested in this area since the end of the 1960s. Harvesting is undertaken according to a regime established by the corresponding county councils such that the number of collectors is restricted. Truffles are harvested with the help of trained dogs. Other zones of the Beteta municipality were also examined along with a nearby area on the banks of the river Guadiela.

This study was based on the collaboration of 14 truffle collectors, who offered their knowledge including the location of several harvesting points and collection data. During several expeditions undertaken from 1994 and 2003 with these

collectors, we determined the plant composition of 433 production sites of *T. melanosporum*. The term 'quemado', translated here as 'burn', is used in Spain to denote such sites because of their burnt appearance: the areas are typically clear of vegetation as a result of the phytotoxicity of *T. melanosporum* (Montachini *et al.* 1972; Papa 1992; Reyna 1999). In most of these burns, *T. melanosporum* was associated with *Quercus faginea* Lam., *Q. ilex* L. subsp. *ballota* (Desf.) Samp., *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco, *P. sylvestris* L., *Cistus laurifolius* L., *Corylus avellana* L., and *Tilia platyphyllos* Scop.

Using these data, we were able to characterise 8 types of habitat in terms of their vegetation, symbiont species of the truffle, physiography, surface stoniness of the soil and yield of *T. melanosporum*. To this end, the slope (% gradient) was determined in a sample of 180 burns using a clinometer (SILVA Clino master). In these burns, stony surface cover was also determined (expressed as the % surface area occupied by stones and gravel).

In a further sample comprised of 208 burns, surface areas were determined in m² and maximum annual carpophore production established through interviews with 14 collectors. The reliability of the information provided by collectors was confirmed by field trips at the time of harvesting over the period 1994 to 2003. Using these two sets of burns, estimation was made of the total yearly yield, the slope and the surface stoniness of the different habitats identified.

In the largest habitat (habitat type 1), we performed a preliminary evaluation of the dasometric variables of the tree symbionts of *T. melanosporum*. To this end, we established two 'forest stations' in which we obtained a set of measurements for specimens of *Quercus faginea* associated with the truffle. These measurements were compared with those obtained for individual *Q. faginea* specimens not associated with *T. melanosporum*. For this comparison, we defined a reference station (forest station 1) comprised of open formations growing on sunny SW-orientated, pronounced slopes (54 %) showing marked surface stoniness (60 %). That is, the conditions were appropriate for *T. melanosporum* but these stands lacked productive burns. The second forest station established showed similar features (slope 45 %; surface stoniness 70 %; orientation SW) yet lacked *T. melanosporum* production.

In both forest stations, the stands of *Quercus faginea* selected were of a size and age representative of the formations of the area. In station 2, we evaluated 3 stands corresponding to the 3 burns associated with *T. melanosporum* production as follows: first we identified the trees possibly responsible for the burn and measured the distance between the two trees furthest apart; then, based on the opinion of an expert collector, we identified the centre of the burn and defined a circle using half the distance between the two trees furthest apart as the radius; and finally we took measurements of all the trees within this circle.

For all the selected trees, the measurements made were tree diameter and height. In the case of sprouts from cut trunks we measured all their diameters, but only the height of the tallest specimen. The variable 'crown diameter' was

measured as a maximum and minimum diameter determined at right angles using a tape measure. In these trees, we also measured the height of the first live branch. Trunk diameters were measured with moveable-arm calipers and tree heights using a Suunto hypsometer. These data were compared using the Statistica program, v. 6 (StatSoft, Inc., Tulsa, OK 1999) to establish whether there were any significant differences in the tree diameters and heights of *Quercus faginea* producing *Tuber melanosporum* compared to those with no truffle production. Before statistical analysis, tree heights were neperian log-transformed to adjust their distribution to the criteria of the parametric statistic. The normal distribution of the two variables was confirmed by the Shapiro-Wilks test.

Results

A series of pronounced slopes (Fig. 1) and mountainous areas was identified in Peralejos and Coto de Belvalle showing several habitats of *Tuber melanosporum*. At these sites, we also collected carpophores of *T. rufum* Pico (red truffle denoted “bordes” in Spanish), *T. aestivum* Vittad., and *T. mesentericum* Vittad. (García-Montero 2000). In mid-slope zones, several rocky scarp and stony areas generated by meteorisation of rock faces were found to generate *T. melanosporum* (Fig. 2). These rocky sites were contiguous with other habitats on soils that were more typical of forests and with riparian formations along the Tajo River.



Fig. 1. Steep banks of the “Parque Natural del Alto Tajo” with several types of habitat that generate high yields of *Tuber melanosporum* (see the flying vultures)



Fig. 2. Mid-slope zones with several rocky scarp and stony that produce *T. melanosporum*

Habitat type 1. Habitats with *Tuber melanosporum* burns located in mono-species *Quercus faginea* formations of the vegetation series *Cephalanthero longifoliae* - *Querceto fagineae* S., with a few isolated specimens of *Quercus ilex* subsp. *ballota*. These formations are fragmented and have a highly variable density of trees, due as much to human activities as to the occurrence of stony soils. Most of the burns occur in open areas and at the outer limits of the stands of *Q. faginea*.

The physiography of the habitat is variable but in line with the general orography of the high Tajo Basin. Truffle sites occur on steep slopes of mean gradient 19 %. Moreover, 24 % of the burns show a slope between 30 and 70 %. Surface stoniness is high and accounts for a mean of 73 % of the cover; 45 % of the burns showing proportions of stones as high as 90-100 % (Tab. 1).

Inventories were established for 134 burns, and surface area and truffle yield estimates were made in 83 of these burns (Tab. 2). Production values were high with maximum yearly yields of 1351 g/burn. We were also able to identify 12 exceptional sites capable of producing 3 to 6 kg/year. Burns were easily identified. Their mean surface area was 8 m², and 11 burns were 20 to 40 m².

In these zones, *Quercus faginea* is also associated with carphophores of *Tuber rufum* and *T. aestivum*. This habitat has a more extensive typology in the study area and we therefore undertook a preliminary statistical comparison between the dasometric variables obtained for *Quercus faginea* trees hosting *Tuber melanosporum* and those not related to truffle production. Through ANOVA, we confirmed that there was no significant relationship between truffle production and the dasometric variables of these trees. Table 3 shows the mean dasometric measurements obtained.

Habitat type 2. Habitats with mixed stands of *Quercus ilex* subsp. *ballota* and *Q. faginea*, in Peralejos and Coto de Belvalle. These mixed woods are small and, compared to habitat 1, the soil is more rocky and clayey and is often more exposed to the sun.

The oaks of these permanent communities are related to more xeric lithologies and occur in an isolated manner in the domain of the association *Cephalanthero longifoliae* - *Querceto fagineae* S. These woods harbour an abundance of shrubs including *Genista scorpius* (L.) DC. subsp. *scorpius*, *Buxus sempervirens* L., *Berberis vulgaris* L. subsp. *seroi* O. Bolós & Vigo, and *Genista mugronensis* Vierh. subsp. *rigidissima* (Vierh.) Fernández-Casas. This habitat also yields *Tuber rufum*.

The physiography of the habitat is variable, with areas of mild topography alternating with more abrupt zones. Its burns occur on marked slopes; the mean slope for the area being 19 %. Further, 18 % of its sites show slopes in the range 35 to 70 %. Surface stoniness is high, representing a mean of 76 % cover and 41 % of the burns have a 90-100 % proportion of stones (Tab. 2).

Thirty eight burns were surveyed in habitat type 2 (Tab. 1). The productivity of these burns was notable, with a

mean annual yield of 743 g/burn. Ten sites showed a truffle production of 1500 to 3000 g/year. Burns were larger than in the other habitats (Tab. 1); their mean surface area being 19 m² with 9 exceptional cases of burns 30 to 150 m². Most burns appeared in open areas and at the margins of the stands.

Habitat type 3. A habitat with isolated burns of *Quercus faginea* and *Q. ilex* subsp. *ballota* in rocky escarpments found in high bank zones or mid-slope areas of rocky shelves. Truffle host trees grow at sites of gentle slope (mean 3 %; maximum 20 %) with high amounts of stones (mean 89 %, Tab. 2). These burns occur on thin soils probably formed by transport and deposition. Vegetation is scarce, and the burns are generally small and scarcely productive.

The 30 burns surveyed showed a maximum production of some 300 g/year on average with some yielding up to 1 kg/year. This habitat type occurs in Peralejos and Belvalle.

Habitat type 4. *Tuber melanosporum* was also associated with formations of *Pinus nigra* subsp. *salzmannii* and *P. sylvestris* including specimens of *Quercus faginea* and even *Q. ilex* subsp. *ballota*. Some of these stands have been repopulated and are therefore more extensive. These pinewoods were located on the highest crests and hills of the area (*Sabino* - *Pineto sylvestris* S).

The pinewoods of this habitat show a variety of soils, from poorly-developed to relatively deep. Its physiography also varies but is generally abrupt. The burns occur on steep slopes of mean 26 %, and 42 % of the burns lie on slopes of 30 to 45 %. Surface stoniness was high, affecting a mean area of 72 % with 42 % of the burns having 90-100 % stones (Tab. 2). In this type of habitat, 106 burns of *T. melanosporum* were identified in Peralejos and Belvalle.

Habitat type 5. This habitat contained riparian formations hosting *Tuber melanosporum* in areas of abrupt topography and ravines. The habitat is comprised of woods of *Corylus*, *Quercus*, and *Tilia* species of the geoserries *Astrantio* - *Coryleto avellanae* S.

Physiography is changing but generally abrupt. Its burns occupy marked slopes of a mean of 22 %, and 17 % show slopes of 40 to 70 %. Surface stoniness is substantial and occupies a mean area of 60 %, with 25 % of burns containing 100 % stones (Tab. 1).

In this habitat, 39 burns were examined and it was possible to estimate the surface area and truffle yields of 27 of them (Tab. 1). The *T. melanosporum* burns appeared alongside specimens of *Quercus faginea*, *Corylus avellana*, and sometimes those of *Tilia platyphyllos*.

The productivity of this type of habitat was considerable. The mean maximum yearly truffle yield was 686 g/burn (Tab. 2). At 6 sites, yields as high as 1200 to 4000 g/year were obtained. The burns were appreciable with a mean surface area of 10 m², 6 of which occupied 20 to 50 m². Habitat 5 occurs in Peralejos and Belvalle.

Table 1. Slope and surface stoniness values for 180 burns in 7 habitats

Habitat	1	2	3	4	5	6	7	Total
Vegetation	<i>Q. faginea</i>	<i>Q. ilex</i> / <i>Q. faginea</i>	<i>Q. faginea</i> (on rocky soil)	<i>Pinus</i> sp.	<i>Tilia</i> sp./ <i>Q. faginea</i>	<i>Corylus</i> sp./ <i>Q. faginea</i>	<i>Cistus</i> sp.	-
no. burns	88	27	11	19	12	3	20	180
% mean slope	19	19	3	26	22	5	15	19
SD	14	15	5	10	18	0	10	13
% max. slope	70	70	20	45	70	5	45	70
% mean stoniness	73	76	89	72	60	33	39	69
SD	25	23	9	27	34	6	13	27
% max. stoniness	100	100	100	100	100	40	70	100

Table 2. Truffle yields and sizes of 208 burns found in 5 habitats

Habitat type	1	2	5	6	8	Total
Vegetation	<i>Q. faginea</i>	<i>Q. ilex</i> / <i>Q. faginea</i>	<i>Tilia</i> sp./ <i>Q. faginea</i>	<i>Corylus</i> sp./ <i>Q. faginea</i>	<i>Q. ilex</i> / <i>J. thurifera</i>	-
area surveyed	Peralejos	Peralejos & Belvalle	Peralejos & Belvalle	Guadiela river	Beteta	-
no. burns	83	38	27	25	35	208
mean yield g/burn	1351	743	686	534	577	925
SD	1322	825	951	586	712	1084
min. g/burn	100	60	30	100	50	30
max. g/burn	6000	3000	4000	1500	3500	6000
mean m ² /burn	8	19	10	4	8	10
SD	8	36	13	4	9	18
min. m ² /burn	1	0.5	1	1	1	0.5
max. m ² /burn	40	150	50	14	50	150

Table 2 provides maximum yearly truffle yields for the habitats identified (habitats 3, 4, and 7 were not included due to a lack of appropriate production data). The eight types of *Tuber melanosporum* habitats defined and characterised in the study area are described below. A summary of the distribution of slopes and surface stoniness of these habitats is provided in Tab. 1.

Table 3. Dasometric analysis of two forest stations representative of the type 1 habitat

	% population	no. trees	dt ^d (cm)	hf ^e (m)	hl ^f (m)	dc1 ^g (m)	dc2 ^h (m)
mean THT ^a	36	8	11.5	5.7	1.5	3.4	4.3
SD			4.1	2.2	0.5	0.4	1.2
mean NTH ^b	64	14	9.9	5.3	2.5	4.8	5.7
SD			4.0	1.6	1.4	0.3	0.8
mean TT ^c	100	22	10.5	5.5	2.0	4.1	5.0
SD			4.0	1.8	1.1	0.8	1.2

Abbreviations: ^a truffle hosting trees; ^b trees not hosting truffles; ^c total tree population; ^d trunk diam; ^e full height; ^f height of the first live branch; ^g max. crown diam; ^h min. crown diam

Habitat type 6. This habitat found on a bank of the river Guadiela is related to riparian geoseries of *Astrantia* - *Coryleto avellanae* S. and is formed by hazel and oak.

Its physiography is mild and its truffle burns appear on practically flat areas of 5 % mean slope. Surface stoniness is low achieving a mean cover of 33 % (Tab. 1). Twenty five *Tuber melanosporum* burns were examined in this habitat associated with *Corylus avellana* and *Quercus faginea*, and estimation made of their surface areas and truffle yields (Tab. 1). Productivity was low, estimated at a mean maximum annual yield of 534 g/burn. Seven points were identified as producing 1000 to 1500 g/year. These burns were smaller than those of the remaining habitats, their mean area being 4 m² with 4 burns measuring 10 to 14 m².

Habitat type 7. This interesting habitat bears *T. melanosporum* burns associated with *Quercus faginea* and *Cistus laurifolius*. This wood belongs to the series *Cephalanthero longifoliae* - *Querceto fagineae* S. whose shrublands include several plants of the association *Genisto scorpii* - *Cistetum laurifolii* Rivas-Martínez (unpublished) (Cruz 1994). These formations were identified in Coto de Belvalle.

The soils of this habitat are of several depths. Its physiography is variable yet not abrupt. Its black truffle burns show a mean slope of 15 %, and 10 % slopes between 30 and 45 %. Surface stoniness is moderate covering 39 % and proportions of 50-70 % stones were shown by 30 % of the burns (Tab. 2).

Of the 26 burns analysed, yields were monitored for 3 years and surface area values determined in 21. These three seasons, however, were not particularly good for the black truffle. The mean annual yield was low at 216 g/burn and only three points yielded 500 to 700 g/year. Burns were small with a mean surface area of 6 m² and 4 cases between 14 and 20 m².

Habitat type 8. A habitat composed of trees of the series *Junipero thuriferae* - *Querceto rotundifoliae* S. The vegetation of these areas is clearly more xeric than the previous habitats. *Quercus ilex* subsp. *ballota* is an Iberian oak proper to fairly warm, dry sites (Castroviejo 1986-1990). Habitat 8 was described in the district of Beteta.

Thirty five burns were surveyed and it was possible to establish surface areas and yields for all of them (Tab. 2). These oakwoods appeared on gentle slopes of intermediate stoniness. Truffle production was moderate, with a mean annual yield of 577 g/burn. Ten points were identified as producing 1000 to 3500 g/year. Burns were appreciable, their mean surface area being 8 m² with 5 between 15 and 50 m².

Discussion

Through the study of 433 burns of *Tuber melanosporum* in the high Tajo Basin, eight habitats were characterised according to their vegetation, symbiont plant species, physiography, surface stoniness, and their truffle production capacity. These habitats

indicate *T. melanosporum* is able to produce fruiting bodies in a wide variety of environmental conditions in symbiotic partnership with 7 plants growing within a 10 km radius (*Quercus faginea*, *Q. ilex* subsp. *ballota*, *Pinus nigra* subsp. *salzmannii*, *P. sylvestris*, *Cistus laurifolius*, *Corylus avellana*, and *Tilia platyphyllos*).

The burns of the black truffle can be observed across topographies which are highly variable, but generally abrupt, with mean slopes in the range 3 % to 26 %, and 44 % of the burns appearing on gradients between 20 and 70 %. The burns of the Alto Tajo generally show high proportions of surface stoniness with mean values ranging from 33 % to 89 %. We identified several sites with 100 % stone cover and high truffle yields (up to 6 kg/year). These stony sites safeguard *T. melanosporum* from the effects of excessive collection and consumption by animals (García-Montero 2000).

The marked variation in the environmental conditions of the 8 habitats of *T. melanosporum* within a relatively small area gives rise to considerable differences in truffle yields. For instance, Tab. 1 shows that the 3 habitats in Peralesjos and Belvalle (within a small distance of each other) show a 50 % difference in their maximum annual yields, while the set of 5 Alto Tajo habitats surveyed vary by as much as 60 % in terms of truffle yields. Thus, within a radius of 10 km, the production of *T. melanosporum* can change substantially among the micro-environments of different habitats, the ecological features of which need to be evaluated as precisely as possible.

Through the comparison of truffle yields among the habitats, it also emerges that the Iberian species *Quercus faginea* and *Q. ilex* subsp. *ballota* are excellent hosts for this highly prized truffle. In particular, the stands of *Q. faginea* in the type 1 habitat gave rise to high production values (Tab. 1). This symbiont species is described in more detail in García-Montero *et al.* (2001). In contrast, *Cistus laurifolius* and *Tilia platyphyllos* do not act as hosts for *T. melanosporum* in the high Tajo Basin region.

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