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Impact of active soil carbonate and burn size on the capacity of the rockrose *Cistus laurifolius* to produce *Tuber melanosporum* carpophores in truffle culture

Luis G. GARCÍA-MONTERO[a,*], *Miguel Á. CASERMEIRO*[b], *José L. MANJÓN*[c], *Isabel HERNANDO*[b]

[a]*Department of Forestry Engineering, U.D. Operaciones Básicas, E.T.S. Ingenieros de Montes, Technical University of Madrid (UPM), Ciudad Universitaria s/n, Madrid 28040, Spain*
[b]*Department of Soil Science, University Complutense of Madrid, Ciudad Universitaria s/n, Madrid 28040, Spain*
[c]*Department of Plant Biology, University of Alcalá, Crtra. Barcelona Km 33, Alcalá de Henares 28870, Spain*

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ABSTRACT

There are very few studies on *Tuber melanosporum* associated with *Cistus* populations. In central Spain, we confirm that *C. laurifolius* shows carpophore production of *Tuber melanosporum*. This study demonstrates a correlation between *T. melanosporum* production and the size of the 41 burns, with burn size explaining 26 % of the variability in carpophore production. However, statistical analysis of the results indicates that average production of 21 burns with *Cistus laurifolius* is 73 % lower than the production of 20 burns associated with *Quercus/Corylus* without *Cistus laurifolius* in this zone, respectively. *C. laurifolius* develops small burns, which has an impact on their carpophore production, and their soils have 92 % less active carbonate than the burns associated with *Quercus/Corylus* in this zone, respectively. The low levels of active carbonate allow *Cistus laurifolius* to grow, but impair *Tuber melanosporum* production. We thus provide a mechanism, based on the inability of *Cistus laurifolius* to grow in highly carbonated soils, which contradicts the well-known fact that *C. laurifolius* might act as a transmitter of *Tuber melanosporum* mycorrhizae. These results indicate that this rockrose can not be considered a useful species for truffle culture. We thus recommend that particular attention should be given to the concentration of active carbonate present in the soil in future studies on the relationship between *Cistus* species and truffles.

Introduction

The most important studies on the ecology of *Tuber melanosporum* (Périgord black truffle) have been carried out in forests and thickets of *Quercus*, *Corylus*, and *Ostrya*, located in the Mediterranean areas of France, Italy, and Spain. It is known that *Tuber melanosporum* can mycorrhize with plants of the genus *Cistus* (rockroses). However, few studies exist on *Tuber melanosporum* associated with natural or cultivated *Cistus* populations. In Italy, a natural association has been recognised between *C. monspeliensis* and *T. melanosporum* in communities that produce carpophores. Mycorrhization, burn formation, and *T. melanosporum* production with *C. incanus* have been confirmed in several natural areas (Fontana & Giovannetti 1979; Granetti *et al.* 2005; Minutoli 1992).

It has been proposed that the influence of *Cistus* in truffle culture is due to the fact that these bushes may assist in the mycorrhization of trees, the survival of the vegetative

* Corresponding author.
E-mail address: luisgonzaga.garcia@upm.es
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apparatus of *Tuber*, and may even cause early carpophore production in the third year of cultivation. Additionally, *Cistus* is capable of growing in poor soils and, due to its small size, allows a greater number of plants per hectare, which has been suggested as a means of incrementing truffle production in intensive cultivation (Chevalier *et al.* 1975; Giovannetti & Fontana 1982; Granetti *et al.* 2005; Manna 1992).

Nevertheless, there is a lack of data on the natural production of *T. melanosporum* in spontaneous association with *Cistus* spp. Neither the small number of growth experiments nor the few field studies available on this topic make it possible to evaluate the usefulness of *Cistus* in truffle culture (Giovannetti & Fontana 1982; Manna 1992, 1999; Minutoli 1992), nor do they provide any hypotheses about causal mechanisms.

We located a wild population of *C. laurifolius* in the central Iberian Peninsula (Alto Tajo Nature Reserve), which has been producing *T. melanosporum* carpophores for the last 25 y (García-Montero *et al.* 2005b,c, 2007b). *C. laurifolius* is a shrub inhabiting formations of *Quercus* and *Pinus*, bleak uplands, steppes and pastures, located on slopes and stony soils in Mediterranean mountains. This species grows in siliceous or decarbonated calcareous soils, with an altitude interval range between 400 and 1900 m (Demoly & Montserrat 1990).

In this study, our goal was to broaden the knowledge of the value of *C. laurifolius* in truffle culture and to provide data on the causal mechanisms governing the ecological relationships between *Cistus* and *Tuber* species. We analysed the productive capacity of *C. laurifolius* compared with other plants with a symbiotic relationship with *T. melanosporum* (*Corylus* and *Quercus* species). This was accomplished by measuring the size of the burns and making a precise record of the production of carpophores over a period of three years. We also wanted to confirm that *C. laurifolius* mycorrhizes easily with *T. melanosporum* in a natural environment. Finally, the impact of soil on the production of *C. laurifolius* burns was studied through an analysis of the carbonate in the soil of 20 *T. melanosporum* burns with and without *C. laurifolius*.

Materials and methods

Study area

The study area is situated in an adjoining series of ravines and deep gorges, located within the Alto Tajo Nature Reserve, on the border between the Belvalle tract and the municipal district of Peralejos de las Truchas (Geographic coordinates: 40° 35′ 49.65″ N; 1° 54′12.78″ W). This study area is in a mountainous region, at an altitude ranging between 1200 m and 1500 m, in a supraMediterranean bioclimatic belt, with a subhumid, shady climate. Average annual precipitation is 797 mm, with low average yearly temperatures (9.7 °C) and very cold winters (coldest month with a mean minimum temperature of between −7 and −4 °C).

In this area we located 41 *Tuber melanosporum* burns in three habitat types sited close together and sharing similar topographic and mesoclimatic conditions (5 km radius). We recorded the surface area (in m^2) and productivity (see below) of all burns studied. The three neighbouring habitats were defined according to their vegetation, symbiotic plants, physiography and stoniness. García-Montero *et al.* (2005b) describe the study area and these habitats in great detail.

Habitat I

This interesting habitat contains *Tuber melanosporum* burns associated with open woods of *Quercus faginea* and *Cistus laurifolius*. This wood belongs to the geobotanical series *Cephalanthero longifoliae–Querceto fagineae*, whose shrublands include several plants of the geobotanical association *Genisto scorpii–Cistetum laurifolii*, dominated by *C. laurifolius* (Cruz 1994). The tree stands and thickets, with an approximate extension of 4 ha, are distributed over several hill slopes. Inventories were established for 21 burns associated with *C. laurifolius*.

Habitat II

Mixed wood composed of *Corylus avellana*, *Quercus faginea*, and some examples of *Tilia platyphyllos*. The habitat contains riparian formations hosting *Tuber melanosporum* in areas of abrupt topography situated at the base of the slopes of ravines along the Tajo River. The woods belong to the geobotanical association *Astrantio–Coryleto avellanae* Inventories were established for ten burns without *Cistus laurifolius*.

Habitat III

Habitat with *Tuber melanosporum* burns located in stands of *Quercus ilex* subsp. *ballota*. This habitat contains open woods of the geobotanical association *Junipero thuriferae–Querceto rotundifoliae* on the top of the slopes. Inventories were established for ten burns without *Cistus laurifolius*.

Truffle productivity

Over three years, all the *Tuber melanosporum* carpophores produced in the 41 burns in the analysis were collected. The burns were monitored by a professional harvester using dogs, and this was the only person harvesting the study area throughout the three years of the study. Truffle production was determined as the maximum annual production in grams of fresh fruiting bodies of *T. melanosporum* per burn. The geographic coordinates of burns are not given in order to maintain the secrecy of the black truffle locations.

Soil analysis

We studied the soil of 20 *Tuber melanosporum* burns belonging to habitats I, II and III. The criteria for selecting these soil samples were the presence/absence of *Cistus laurifolius* and the maximum annual production of *Tuber melanosporum* carpophores (Table 1). For each soil, we studied the first 30 cm of the profile, as black truffles usually bear fruit in this range (Verlhac *et al.* 1990). Sampling was completed according to FAO (1990). The following determinations were made: pH (1:2.5 soil suspension/water), total calcium carbonate (equivalent carbonate: by volume of hydrochloric acid) following the methods of the ISRIC (1995), and active carbonate (carbonate