Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems
http://dx.doi.org/10.5424/fs/2014232-04900

Forest Systems 2014 23(2): 307-316
ISSN: 2171-5068
eISSN: 2171-9845

Phytosanitary problems detected in truffle cultivation in Spain. A review

Maria Martin-Santafe[1,2]*, Victor Perez-Fortea[3], Pedro Zuriaga[3] and Juan Barriuso[1]

[1] Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Gobierno de Aragón. Avda. Montañana, 930. 50059 Zaragoza, Spain. [2] Centro de Investigación y Experimentación en Truficultura de la Diputación de Huesca. Polígono Fabardo, s/n. 22430 Graus (Huesca), Spain. [3] Laboratorio de Sanidad Forestal. Gobierno de Aragón. Servicio Provincial de Agricultura, Ganadería y Medio Ambiente. C/ Agustín Planas Sancho, 10, 3º. 44400 Mora de Rubielos (Teruel), Spain

Abstract

Aim of study: In black truffle plantations, many factors are driving the emergence of new pests and diseases which in forestry areas go unnoticed. Usually, the incidence of most of them is low. Nevertheless, in specific cases, some of them are capable of causing irreversible damage that could endanger the harvest or even tree survival.

Area of study: This paper presents an in-depth study of the pests and diseases more frequently associated with truffle plantations in the region of Aragón (Spain). Damages have been arranged into the main production stages: nursery, plantations and fruiting bodies.

Material and methods: The data used in this work come from the technical enquiries from truffle growers to researchers and technical staff, as well as from field visits undertaken by those work teams.

Sampling methodology has been carried out following standard procedures. Insects were collected with the traps commonly used in entomology works.

Main result: More than 50 damages have been described in this paper. Some of them are capable of weakening the plants and other can even kill them. Mycorrhizal competitor fungi have also been considered in this paper. These organisms do not cause real phytosanitary problems, but they can lead to important economic losses.

Research highlights: Researches, truffle hunters and managers must be alert in the face of the possible occurrence of potentially dangerous organisms. The final aim: being able to take action in an efficient way in the case of a pest outbreak.

Key words: *Tuber melanosporum; Quercus;* parasites; pests; diseases.

Introduction

Black truffle is the fruiting body of a hypogeous ascomycete (*Tuber melanosporum* Vittad.) which establishes symbiotic relationships with different spermatophyte species, mainly of the genus *Quercus*. The main host trees for black truffle in Spain are *Quercus ilex* L. subsp. ballota (Desf.) Samp., *Quercus faginea* Lam., *Quercus coccifera* L., *Quercus humilis* Mill., *Tilia platyphyllos* Scop. and *Corylus avellana* L. (Etayo *et al*., 1999).

Nowadays 10,000 ha are estimated to be used for truffle culture, of which approximately 6,000 ha are in the region of Aragón (North East Spain). Annual growth is estimated at 500 ha (Reyna *et al.*, 2013). Due to the high profitability of these plantations and to the support of the public authorities, Spain is becoming a world leader in this activity. But this is not the only marketable truffle in Spain. Other truffles, as *Tuber aestivum* Vittad., or *Tuber brumale* Vittad. are also collected and traded (De Román and Boa, 2004). However, their lower cost and the lack of knowledge about their culture are directing all efforts of truffle growers and researchers towards an increase of *T. melanosporum* production.

As the trend to monoculture grows and plantation densities increase, the quantity of pests associated with

* Corresponding author: mmartinsa@aragon.es
Received: 30-08-13. Accepted: 26-02-14.

This work has 32 Supplementary Figures that do not appear in the printed article but that accompany the paper online.

truffle plantations keeps rising. In fact, insects that lived in balance in the natural environment have turned to pests in recent years.

This paper presents a comprehensive study of pests and diseases detected in truffle plantations in Spain.

Material and methods

Study area involves truffle plantations and forest, mainly in the region of Aragón. This is a spanish region with optimal eco-climatic conditions for black truffle development. This fact is reflected in the total number of truffle plantations and in the high yields that are obtained from this activity.

Sampling methodology has been carried out following standard procedures. Insects were collected with the traps commonly used in entomology works. Traps include Malaise trap (Townes, 1972), handmade budget sleeves and yellow sticky cards (CROMATOL® Halper Industria química, S.L. Pontevedra, Spain). Visual inspection of damaged trees has been also used to identify the pathogenic causal agent.

After trapping, samples were carried to the laboratory. Mainly, the works carried out by De Liñán (1998), Fraval (1998), Agrios (1998) and a database from the University of California (http://www.ipm.ucdavis.edu/) were used to identify all the insects appearing in plantations, forests and nurseries.

The results of this work are arranged in the three main stages of truffle culture: nursery, plantation and fruiting bodies. Mycorrhizal competitor fungi have also been considered in this paper. These organisms do not cause real phytosanitary problems, but they can lead to important economic losses. Ectomycorrhizal types were identified by anato-morphological features according to Agerer (2006) and Agerer and Rambold (2004-2013).

Results and discussion

Pests associated with nurseries

As for plant production in nurseries, pathogenic fungi, competitor fungi with *T. melanosporum* mycorrhizas and arthropods have been detected (Table 1).

The presence of powdery mildew *(Microsphaera alphitoides* Griffon & Maublanc) (Suppl. Fig. S1 [pdf online]) is common in plants established in the field. Furthermore, it also appears quite frequently on oaks [*Quercus robur* L., *Quercus petraea* (Matts.) Liebl., *Q. faginea, Q. humilis,* etc.] during the nursery phase. Its proliferation is normally related to excess moisture, and it is treated with sulfur or its derivatives (Zambonelli, 1993). This procedure, although effective, must be carried out with the necessary precautions when working with inoculated plants, as this antifungal treatment can endanger the correct mycorrhization process. Days when high temperature occurs should be avoided, since a phytotoxicity effect can be produced.

Other fungi in nurseries are able to compete with *T. melanosporum* mycorrhizae. This is the case of mycorrhizal fungus *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček y Kubička (Suppl. Fig. S2 [pdf online]). This ascomycete is a pioneer fungus able to form mycorrhizae faster than *T. melanosporum*. Thus, it can displace the latter from its host plant as it infects root tips. *S. brunnea* limits the proper plant mycorrhization, and therefore its quality, sometimes even causing economic losses (Sánchez, 2012). It is difficult to eradicate when present in greenhouses because its control is achieved by managing moisture and temperature conditions (Sánchez *et al.,* 2014). Nevertheless, this species shows low competition under field conditions and it does not cause problems in plantations (Hall *et al.,* 2007).

Other ectomycorrhizal fungi can be found in nurseries. This is the case of *Tuber albidum* Complex (Suppl. Fig. S3 [pdf online]), and *Trichophaea woolopheia* (Cooke & W. Philips) Boud. (Suppl. Fig. S4 [pdf online]). Their presence is usually due to the use of poorly disinfected soil from forestry areas when preparing substrate (Garcia-Barreda, 2011). Other species of the genus *Tuber*, such as *T. brumale,* are sometimes detected. This situation is due to a mistake in the inoculum selection, although this is not common.

Different arthropods are relatively common in mycorrhizal plant production nurseries, although damages are usually not relevant and they do not weaken the plants excessively. They include the Coleoptera *Otiorhynchus sulcatus* Fabricius (Casteels and De Clercq, 1988) (Suppl. Fig. S5 [pdf online]), Eriophyid mites (Suppl. Fig. S6 [pdf online]) as *Cecidophes* sp. Nalepa, and *Aceria ilicis* Canestrini, Lepidoptera as *Phyllonorycter belotella* Staudinger, the Homoptera *Phylloxera quercus* Boyer de Fonscolombe (Suppl. Fig. S7 [pdf online]) or different species of aphids.

Due to their large size and high caloric content in lipids and carbohydrates, acorns are a good source of food. Therefore it is easy to find in them certain insects such as the weevil *Curculio elephas* Gyllenhal (Bonal *et al.,* 2007) (Suppl. Fig. S8 [pdf online]), *Cur-*

Table 1. Diseases or competitors present in each truffle cultivation stage. Their identification, incidence, danger risk and impact when the problem occurs are shown for each pest

Stage	Disease/Competitor	Identification	Incidence	Danger risk	Impact when the problem occurs
Nursery	*Microsphaera alphitoides*	Leaves covered with a white mass of mycelia and conidia. Discoloration, necrosis, uneven shoot growth	High	Low	May delay the growth of young plants and may kill tree seedlings
	Competitor fungus				
	Sphaerosporella brunnea (anamorph *Dichobotrys brunnea*)	Presence of mycorrhizae. In severe cases, appearance of fruiting bodies	High	Medium	Rejection of the batch depending on the method used for seedling quality evaluation
	Tuber albidum Complex	Presence of mycorrhizae	Low	High	Rejection of the batch
	Trichophaea woolopheia	Presence of mycorrhizae	Low	Medium	Rejection of the batch depending on the method used for seedling quality evaluation
	Tuber brumale	Presence of mycorrhizae	Low	High	Rejection of the batch
	Otiorhynchus sulcatus	Leaves with scalloped edges eaten by adults Presence of larvae feeding on roots	Low	High	Weakness and subsequent death of affected plants
	Cecidophes sp.	Malformations in shoots and buds and disordered growth in them	Low	Low	Weakness and malformations in affected plants. Decrease of the seedling economic value due to poor appearance
	Aceria ilicis	Erinea in leaves. The affected leaves initially turn into a yellowish colour that later evolves into dark brown	High	Low	Seedlings usually do not suffer excessively with this pest. Decrease of the seedling economic value due to poor appearance
	Phyllonorycter belotella	Mines in the upper surface of leaves	Low	Low	Weakening of the seedling. Decrease of the seedling economic value due to poor appearance
	Phylloxera quercus	Yellow and brown spotting of leaves. General browning of the foliage and premature leaf fall	Medium	Low	Normally of no importance but persistent attacks on young trees reduce plant vigour
	Aphids	Presence of aphids in shoots, frequently accompanied by sooty mould	High	Low	Weakening of the plant
	Curculio elephas *C. glandium*	Holes in the acorns. Compact droppings inside acorns	Medium	Medium	Economic losses due to germination failure
	Cydia fagiglandana	Holes in the acorns. Granulated droppings inside acorns	Medium	Medium	Economic losses due to germination failure
	Apodemus sylvaticus *Microtus* sp.	Extraction of part of the potting mix from the seedling containers. Absence of acorns in containers	Low	Medium	Economic losses due to germination failure
Plantations	*Kermes ilicis* *K. vermilio*	Presence of females on the twigs and branches	Medium	Medium	Weakening of the plant even death in case of persistent attacks
	Targionia vitis	Presence on stems and branches, especially under the bark	Low	Low	Weakening of the plant
	Coroebus florentinus	Yellowish brown branches, evolving later into a reddish tone. Presence of galleries that spiral around the branch	Low	Low	Imbalance between aerial and root parts due to galleries, which interrupt the sap flow. Weakening of the plant. Brittle branches

Table 1 (cont.). Diseases or competitors present in each truffle cultivation stage. Their identification, incidence, danger risk and impact when the problem occurs are shown for each pest

Stage	Disease/Competitor	Identification	Incidence	Danger risk	Impact when the problem occurs
	Cossus cossus	Presence of sawdust and/or droppings. Presence of larvae and galleries under the bark	Low	Medium	Imbalance between aerial and root parts due to galleries, which interrupt sap flow. Weakening of the plant. Brittle branches
	Zeuzera pyrina	Presence of newly hatched larvae in young twigs, to be found later in larger branches or trunks	Low	Medium	Imbalance between aerial and root parts due to galleries, which interrupt sap flow. Weakening of the plant. Brittle branches
	Lymantria dispar	Insect egg depositions on vertical or overhanging surfaces, usually trunks, and never on the floor	Low	Medium	Severe defoliation. Weakening of the plant, leading sometimes to disease and/or death
	Tortix viridiana	Symptoms visible in the tree crowns including complete defoliation when larvae are found in large numbers	Low	Low	Weakening of the plant. Reduction of acorn production
	Euproctis chrysorrhoea	Defoliation. Gregarious and highly urticating larvae, which spin a very visible web around them	Low	Low	Defoliation caused by larvae, which eat the buds and young leaves. Weakening of the plant
	Lachnaia hirta	Presence of larvae feeding on sprouts	Medium	Low	Weakening of the plant. Malformations
	Labidostomis spp.	Presence of adults in the tree crown	Low	Low	Defoliation, decreased flowering
	Polydrusus setifrons	They feed on parenchyma, leaving the leaf veins intact	Medium	Low	Weakening of the plant. Reduction of plant vigour
	Aceria ilicis	Erinea in leaves. The affected leaves initially turn into a yellowish colour that later evolves into a dark brown	High	Low	Slightly deformed leaves. Gall-like thickenings and deformations in inflorescenses
	Aphids	Presence of aphids in shoots, frequently accompanied by sooty mould	High	Low	Weakening of the plant
	Phylloxera quercus	Yellow and brown spotting of leaves. General browning of the foliage and premature leaf fall	Medium	Low	Normally of no importance in old plantations, but persistent attacks on young trees reduce plant vigour
	Galls	Presence of galls, malformations or thickenings with different shapes	Medium	Low	Malformations and alterations in twigs, stems, leaves and catkins
	Brenneria quercina	Exudates on stems, branches, twigs or acorns	Low	Low	Disturbance of the vegetative growth process
	Vesperus sp.	Reddish-brown coloration of the plant. Presence of larvae in affected plants	Low	Medium	Death of the plant
	White worms: *Melolontha* sp. *Anoxia* sp.	Presence of larvae in root system. Reddish-brown coloration of the plant	Low	High	Death of the plant
	Sus scrofa and other mammals	Torn and chewed young plants. Soil profile disturbance	High	High	Death of roots, decreasing of truffle production
	White rots: *Armillaria mellea* *Rosellinia necatrix*	Vigour decline, yellowing foliage, reduced leaf size and number. Mycelium in roots	Low	High	Weakening and even death of the plant
	Taphrina kruchii	Smaller yellowing leaves. Short grouped twigs	Low	Low	Falling of the anomalous leaves
	Brenneria quercina	Exudates on stems, branches, twigs or acorns	Low	Low	Disturbance of the vegetative growth process

Table 1 (cont.). Diseases or competitors present in each truffle cultivation stage. Their identification, incidence, danger risk and impact when the problem occurs are shown for each pest

Stage	Disease/Competitor	Identification	Incidence	Danger risk	Impact when the problem occurs
	Botryosphaeria stevensii (anamorph *Diplodia mutila*)	Groups of chlorotic leaves on some branches	Low	Low	Death of twigs and branches
	Candidatus phytoplasma	Presence of tumor malformations in branches and trunk	Still unknown	Still unknown	Weakening of the plant, even death if tumors affect the trunk
	Competitors (fungal complex)				
	Trichophaea woolhopeia	Presence of mycorrhizae	High	Low	Colonization of root tips preventing *Tuber melanosporum* presence in them
	Pisolithus arhizus	Presence of mycorrhizae. In some cases, appearance of fruiting bodies	Medium	Low	Colonization of root tips preventing *Tuber melanosporum* presence in them
	Quercirrhiza squamosa	Presence of mycorrhizae	Medium	Low	Colonization of root tips preventing *Tuber melanosporum* presence in them
	Tuber brumale	Presence of mycorrhizae. In some cases, appearance of fruiting bodies	Low	High	Production of a non-desirable truffle in the plantations
Fruting bodies	*Leiodes cinnamomea*	Presence of galleries and larvae in truffles	High	High	Truffle devaluation, decrease of truffle weight, rot of fruiting bodies
	Helomyza tuberivora	Presence of galleries and larvae in truffles	High	High	Truffle devaluation, decrease of truffle weight, rot of fruiting bodies
	Megaselia sp.	Presence of galleries and larvae in truffles	Low	High	Truffle devaluation, decrease of truffle weight, rot of fruiting bodies
	Drosophila funebris	Presence of galleries and larvae in truffles	Low	High	Truffle devaluation, decrease of truffle weight, rot of fruiting bodies
	Pleurophorus sp.	Presence of insects in truffles	Low	Low	Spreading of microorganisms that cause truffle rot
	Ommatoiulus sabulosus	Presence of insects in truffles	Low	Low	Spreading of microorganisms that cause truffle rot

culio glandium Marsham or the Lepidoptera *Cydia fagiglandana* Zeller (Torres Villa *et al.,* 2008). These insects can cause an important decrease in acorn germination (Soria *et al.,* 1996).

Attacks of mice (*Apodemus sylvaticus* L.) and voles (*Microtus* sp. Schrank) have been rarely detected in seedbeds. A wrong management in nurseries can lead to abiotic damages caused by frosts, water stress or chlorosis due to a nutrient deficiency in the substrate. On the other hand, an inadequate nursery location that leads to achieving a poor seedling hardening may endanger plant survival in the field (Mollá *et al.,* 2003).

Pests associated with truffle plantations

In recent years, different diseases have appeared in truffle plantations (Table 1). Some of them are capable of weakening the plants and other can even kill them.

Damages identified in the crown

The scale insects *Kermes vermilio* Planch. and *Kermes ilicis* L. (Suppl. Fig. S9 [pdf online]) are capable of causing significant damages in branches and trunks. Due to their high incidence, these Homoptera are a real problem in Spanish plantations. Like all sucking insects, direct damages are associated with sap suction. These scale insects weaken the attacked tree, causing a progressive yellowing in leaves and terminal branches, and thus reducing the amount of green shoots (Malumphy, 2008; Spodek and Ben-Dov, 2012). Damages caused by a massive attack of these insects usually do not endanger plant survival, but they can

Figure 1. Larva of *Zeuzera pyrina* feeding on a *Quercus ilex* branch.

induce a total foliage loss (Malumphy, 2008; Martín Bernal *et al*., 2002). Their mobility is not high, so wind and birds must have an important role in the spreading of this insect (García Marí *et al*., 1989).

Targionia vitis Sign. (Suppl. Fig. S10 [pdf online]) is another scale insect which shows a lower incidence. Nonetheless, it is still capable of inducing a weakening in truffle trees.

The Buprestidae *Coroebus florentinus* Herbst (Suppl. Fig. S11 [pdf online]) bores galleries that spiral round the branch inner bark and interrupt sap flow, which causes it to dry out. It also causes an imbalance between aerial and root parts.

Cossus cossus L. and *Zeuzera pyrina* L. (Fig. 1) (Cossidae) can feed on young shoots. If an attack is conducted on the second or third year after plantation, the tree may die.

The proliferation in recent years of some tumor malformations (Suppl. Fig. S12 [pdf online]) appearing in branches and trunk in evergreen oaks (*Q. ilex*) deserves a special mention. Symptoms begin with a chlorotic pattern in younger leaves, followed by defoliation. Oversized malformations develop wherever there are open wounds in the bark. Over time, there is a general weakening of the tree and in some cases it results in death. Recent studies show that a *Candidatus* Phytoplasma is the cause of this disease (Martín *et al.*, 2012).

Within the defoliating complex there is a great number of phytophagous insects. Most of them are Lepidoptera. Three of the most voracious insects, although rare in truffle plantations, are *Lymantria dispar* L. (Suppl. Fig. S13 [pdf online]), *Tortrix viridiana* L. (Suppl. Fig. S14 [pdf online]) and *Euproctis chrysorrhoea* L. (Suppl. Fig. S15 [pdf online]). *L. dispar* and *E. chrysorrhoea* are two polyphagous species (Frago *et al.,* 2010; Schultz and Lechowicz, 1986), while *T. viridiana* is monophagous on *Quercus* but polyphagous within the taxonomic limits of this genus (Du Merle, 1998). In young trees belonging to *Q. ilex* species, a complete defoliation can cause the death of the tree.

There are many other species typically present in plantation tree canopies. However, they keep low population levels and they only are capable of causing real damages when a population explosion occurs. This is the case of Chrysomelidae *Lachnaia hirta* Fabricius*, Agelastica* sp. L. and *Labidostomis* spp. Dejean (Suppl. Fig. S16 [pdf online]) and the weevil beetle *Polydrusus setifrons* Jacquelin du Val.

The presence of Eriophyid mites in oaks is very common, mainly *Aceria ilicis* Canestrini. This mite causes erinea in leaves. The affected leaves initially turn into a yellowish colour that later evolves into a dark brown.

The presence of aphids (Suppl. Fig. S17 [pdf online]) in truffle plantations is frequent. They may cause a weakening of the plant or a leaf mass decrease if the tree is young. These insects are frequently accompanied by sooty mold.

Phylloxera quercus usually appears in *Q. faginea, Q. robur* and *Q. ilex.* Its symptoms consist of yellow spots on leaves coinciding with suction points. These spots, over time, turn to brown and can even cause leaf drop.

Galls (Suppl. Fig. S18 [pdf online]) are defined as an evolutionary response that many vegetables show to the action of different specific organisms. Many species are capable of inducing these structures, such as fungus, bacteria or arthropods. This latter group is the largest, and it includes mainly species of the Cynipidae family. More than 140 Cynipidae species are gall formation inducers. Among them, approximately 70% are associated to trees belonging to the *Quercus* genus (Nieves-Aldrey, 1998). Galls damages are not usually serious, except in those cases in which an imbalance in the ecosystem prevents hyperparasites from controlling the pest population efficiently. Some of the most frequent species in the truffle plantations of Aragón are: *Neuroterus quercusbaccarum* L.*, Neuroterus numismalis* Fourcroy*, Andricus quercusramuli* L.*, Andricus quercustozae* Bosc*, Plagiotrochus quercusilicis* Fabricius and *Dryomyia lichtensteinii* F. Löw (Suppl. Fig. S19 [pdf online]).

Damages caused by the bacteria *Brenneria quercina* (Hildebrand and Schroth) Hauben *et al.* (Suppl. Fig. S20 [pdf online]) are very characteristic. Fruits have an abnormal development with sap exudation (drippy nut disease) (Perez-Izquierdo and Pulido, 2013). It also produces in species of the genus *Quercus*, small longitudinal lesions of a few centimetres on the trunk bark which produce exudates, especially during spring and autumn.

Taphrina kruchii (Vuill.) Sacc. (Suppl. Fig. S21 [pdf online]) is found mainly in evergreen oaks (*Q. ilex*). It produces dense clusters of twigs known as "witches' brooms".

Botryospheria stevensii Shoemaker (anamorph *Diplodia mutila* Fr. Mont.*)* (Suppl. Fig. S22 [pdf online]) is a fungus that causes death of twigs and branches. First symptoms consist of groups of chlorotic leaves. After this, leaf colour becomes brown. In the branches, the bark becomes necrotic, and longitudinal wounds appear. Below the diseased bark, wood colour degenerates into a reddish brown. Attacked trees show twigs with dry tips and branch dieback.

Damages identified in the tree root system

Several arthropod species are capable of causing lesions in roots. Among them, the following must be highlighted: the Curculionidae *Otiorhynchus sulcatus,* the Cerambycidae *Vesperus* sp. Dejean, different Melolonthidae of the genus *Melolontha* Fabricius (Suppl. Fig. S23 [pdf online]) and *Anoxia* Laporte, and Elateridae known as "click beetle". All these insects can cause severe injuries when the plant is young, even death. Attacks take place during larval stage, in which the individuals are equipped with very powerful jaws.

However, not only arthropods can cause damages to the root system. Ascomycete fungus *Rosellinia necatrix* Berl ex. Prill and Basidiomycete *Armillaria mellea* (Vahl) P. Kumm (Suppl. Fig. S24 [pdf online]) cause wood decay. The origin of this problem usually resides in a preceding woody crop. It is associated to water excess, generally due to a shallow water table (Ono, 1970; Ayres and Boddy, 1986).

An extremely serious problem found in the evergreen oak savannas (dehesas) from Andalucía and Extremadura (Spain) (Sánchez *et al.,* 2002) but still not detected in truffle plantations in Aragón is the Oomycete *Phytophthora cinnamomi* De Bary. It occurs when the plant has suffered water stress in the presence of this organism.

P. cinnamomi and *B. quercina* have both been related to the oak decline in Spain (Soria *et al.,* 1997). This is a very serious disease that is widely spread in Spanish holm dehesas. Fortunately, it has not yet become a problem in truffle plantations.

Another species of this genus, *P. ramorum*, has proven to be infective under controlled conditions on *Quercus* (Moralejo et al., 2009). Nevertheless it has not yet been demonstrated its presence on *Quercus* in Spain. Care in handling plant nursery is essential to prevent its occurrence in plantations.

Due to the uncontrolled proliferation of wild boars (*Sus scrofa* L.) in Spain, problems generated by this species have increased in recent years, mainly in wild truffle stands. This fact forces truffle growers to fence their fields when establishing the plantation. These mammals dig in the ground leaving the roots exposed, causing a great loss of root mass. Voles (Suppl. Fig. S25 [pdf online]) and mice also cause root damage. These rodents cause wounds in the root neck, and may lead to tree death. However, they play an important role in the dispersion of spores (Frank *et al.,* 2008).

Otherwise, ectomycorrhizal species *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud**.,** *Pisolithus arhizus* (Scop.) Rauschert (Suppl. Fig. S26 [pdf online]), *Quercirrhiza squamosa* Palfner & Agerer (Suppl. Fig. S27 [pdf online]) and *Tuber brumale* Vittad. (Suppl. Fig. S28 [pdf online]) have been considered in this research work. These fungi are the most common in truffle plantations and belong to the mycorrhizal courtship of *T. melanosporum*. However, the role of these species in black truffle fruiting is still unknown nowadays. Several works (De Román and De Miguel, 2001; Reyna *et al.,* 2006; Rubini *et al.,* 2011; De Miguel *et al.,* 2014) report the frequent identification of these species in truffle plantations, but they still have not been related to the life cycle of *T. melanosporum*.

Pests associated with fruiting bodies

To complement this study, problems in fruiting bodies have been identified (Table 1). In this sense, insects and other animals have a dual role. On one hand, they behave as predators that feed on truffles and, on the other hand, they spread the spores.

Two of the main problems in truffle cultivation nowadays are truffle beetle (*Leiodes cinnamomea* Panzer) and truffle fly (*Helomyza tuberivora* R.-Desv.).

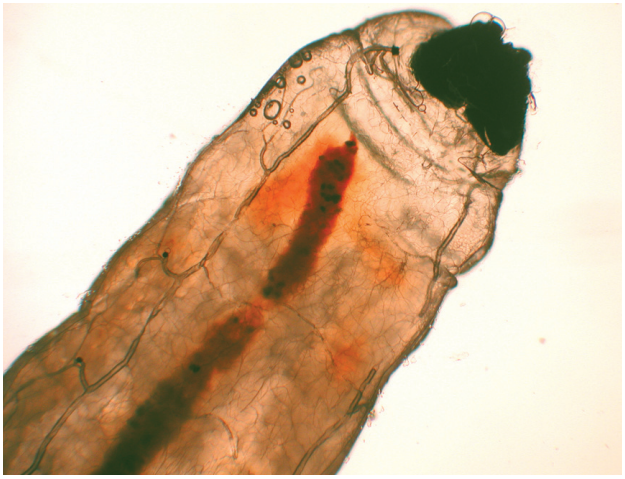

Figure 2. Digestive tract of the truffle fly *Helomyza tuberivora* containing black truffle spores.

Its presence in truffle fruiting bodies is proven since the early twentieth century. Pradel in 1914 and Arzone in 1971, exposed the existing problems with these arthropods, but damages caused by these species are of increasing concern among Spanish truffle growers since the intensification of truffle orchards. On one hand, galleries of larvae cause product depreciation as consumers perceive that larvae and their faeces may be present. And, on the other hand, larva feeding causes a decrease in truffle weight (Barriuso *et al.*, 2012).

H. tuberivora (Fig. 2) has been detected both in *T. melanosporum* and *T. aestivum* carpophores. Their larvae, and therefore their galleries, are generally smaller in size. However, there are usually a high nu mber of larvae feeding on a single truffle. This can reduce up to 40% the fresh weight of the fruiting body. Quality loss is unavoidable because a fast putrefaction and enzymatic degradation occurs throughout the gleba (Rivera *et al.*, 2010).

L. cinnamomea (Suppl. Fig. S29 [pdf online]), is a species strictly linked to *Tuber* species mainly *T. melanosporum* (Pacioni *et al.*, 1991). The proliferation of this beetle in truffle plantations is very high, raising concern among truffle growers and managers. The damages are severe, although putrefaction of the specimens is slower than in the previous case.

There is no effective treatment for these pests. Only cultural actions are carried out to reduce the populations of these insects. A superficial tillage (30-40 cm) to prevent truffle development in the first centimeters and the collection in the plantations of truffles from species different to *T. melanosporum* are the two recommended measures to prevent the spread of these insects.

Other flies, as *Megaselia* sp. Rondani (Suppl. Fig. S30 [pdf online]) and *Drosophila funebris* Fabricius, have also been detected in truffle sporocarps. They have been previously identified as pests of cultivated mushrooms (Gnaneswaran and Wijayagunesakara, 1999).

Other insects have been detected in carpophores, such as *Pleurophorus* sp. Mulsant (Suppl. Fig. S31 [pdf online]) or the myriapod of the Class Diplopoda *Ommatoiulus sabulosus* L. (Suppl. Fig. S32 [pdf online]). These are usually detritivorous and coprophagous species, so their presence in fruiting bodies is due to the prior presence of galleries from other parasites.

Conclusions

The intensification of truffle plantations in Spain has led to the emergence of a large number of pests and diseases associated with this crop. Nevertheless, few of them can be considered dangerous or capable of causing irreversible damage to plantations.

Among all of them *Kermes* sp. and the tumor malformations recently identified as a *Candidatus* Phytoplasma are highlighted. The ectomycorrhizal fungus *S. brunnea* is a problem in nurseries that produce mycorrhizal plants because it lowers *T. melanosporum* colonization and keeps seedlings from meeting quality standards.

Choosing an inappropriate acorn provenance and the use of species not adapted to the plantation area are causing in some cases the plant inadequacy, which leads to poor growth and, in extreme cases, tree death (Andivia *et al.,* 2012; Del Campo *et al*., 2010).

The role of research in phytosanitary issues is clear: potentially dangerous organisms must be identified including their biology, etiology and control measures. The final aim: being able to take action in an efficient way in the case of a pest outbreak. Truffle culture is an organic crop (Reyna*,* 2012). To try and follow this philosophy, any action performed on a truffle plantation to eliminate harmful organisms should be conducted according to the principles of organic farming. Whenever possible, agronomic, biological, and mechanical methods must be applied, as opposed to the use of chemical substances. The search for balance in the ecosystem is an objective to be achieved, trying to promote the existence of native auxiliary fauna and ensuring a proper plant development.

Acknowledgments

This research was supported by INIA (RTA2010-00070-C02).

Authors also want to thank Luz Cocina for her early revision of the manuscript, and to the owners of the plantations who have given us their selfless help.

References

Agerer R, 2006. Fungal relationships and structural identity of their ectomycorrhizas. Mycol Prog 5: 67-107.

Agerer R, Rambold G, 2004. DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, Germany. [5 January 2013].

Agrios G, 1998. Fitopatología. Ed Limusa, México. 838 pp.

Andivia E, Fernández M, Vázquez-Piqué J, Alejano R, 2012. Two provenances of *Quercus ilex* ssp. *Ballota* (Desf) Smp. nursery seedlings have different response to frost tolerance and autumn fertilization. Eur J Forest Res 131: 1091-1101.

Arzone A, 1971. Reperti ecologici ed etologici di *Liodes cinnamomea* Panzer vivente su *Tuber melanosporum* Vittadini (Coleoptera Staphylinoidea). Centro di Entomologia Alpina e Forestale del Consiglio Nazionale delle Ricerche 158: 317-357.

Ayres PG, Boddy L. (eds), 1986. Water, fungi and plants. Cambridge University Press, Cambridge. 413 pp.

Barriuso J, Martín M, Sánchez S, Palazón C, 2012. Plagas y enfermedades asociadas al cultivo de la trufa. In: Truficultura. Fundamentos y técnicas (2) (Reyna S, ed). Ed Mundi-Prensa, Madrid. pp: 275-301.

Bonal R, Muñoz A, Díaz M, 2007. Satiation of predispersal seed predators: the importance of considering both plant and seed levels. Evol Ecol 21: 367-380.

Casteels H, De Clercq R, 1988. Observations on weevil pests in nurseries. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 53: 1169-1174.

De Liñán C. (coord), 1998. Entomología agroforestal. Insectos y ácaros que dañan montes, cultivos y jardines. Ed Agrotécnicas SL. España. 1309 pp.

De Miguel AM, Águeda B, Sánchez S, Parladé J, 2014. Ectomycorrhizal fungus diversity and community structure with natural and cultivated truffle hosts: applying lessons learned to future truffle culture. Mycorrhiza.

De Román M, De Miguel AM, 2001. Identification and description of natural mycorrhizae in a potential truffle-occurring area in Navarra (Spain). Actes du V Congrès International Science et Culture de la Truffe, Aix-en-Provence, France. pp: 8.433-8.436.

De Román M, Boa E, 2004. Collection, marketing and cultivation of edible fungi in Spain. Micol Apl Int 16(2): 25-33.

Del Campo A, Navarro R, Ceacero C, 2010. Seedling quality and field performance of commercial stocklots of containerized holm oak (*Quercus ilex*) in Mediterranean Spain: an approach for establishing a quality standard. New Forests 39(1): 19-37.

Du Merle P, 1998. Egg development and diapause: ecophysiological and genetic basis of phenological polymorphism and adaptation to varied hosts in the green oak tortrix, *Tortrix viridiana* L. (Lepidoptera: Tortricidae). J Insect Physiol 45: 599-611.

Etayo ML, De Miguel AM, De Román M, 1999. Ectomycorrhizae occurring in hazel (*Corylus avellana* L.), oak (*Quercus faginea* Lam.) and evergreen oak [*Quercus ilex* subsp. *ballota* (Desf.) Samp.] in a cultivated bed along 4 sampling years. Publicaciones de biología. Universidad de Navarra. Serie botánica 12: 13-22.

Frago E, Guara M, Pujade-Villar J, Selfa J, 2010. Winter feeding leads to a shifted phenology in the browntail moth *Euproctis chrysorrhoea* on the Evergreen strawberry tree *Arbutus unedo*. Agr forest entomol 12: 381-388.

Frank JL, Anglin S, Carrington EM, Taylor DS, Viratos B, Southworth D, 2008. Rodent dispersal of fungal spores promotes seedling establishment away from mycorrhizal networks on *Quercus garryana.* Botany 87(9): 821-829.

Fraval A, 1998. Encyclopédie des ravageurs européens [on line]. HYPPZ, HYP3. INRA, (France). Available in http://www7.inra.fr/hyppz/ [4 February 2014].

Garcia-Barreda S, 2011. Avances en la selvicultura de bosques mediterráneos productores de trufa negra. Doctoral thesis. Universidad de Valencia, Spain.

García-Marí F, Costa J, Ferragut F, Laborda R, 1989. Plagas agrícolas. Ácaros e insectos exopterigotos. Departamento de producción vegetal, Universidad politécnica Valencia (Spain), Servicio de publicaciones. 209 pp.

Gnaneswaran S, Wijayagunesakara NP, 1999. Survey and identification of insects pests of oyster mushroom (*Pleurotus ostreatus*) culture in central province of Sri Lanka. Trop Agr Res Ext 2(1): 21-25.

Hall I, Brown G, Zambonelli A, 2007. Taming the truffle: The history, lore, and science of the ultimate mushroom. Timber Press. 304 pp.

Malumphy CP, 2008. First british outbreak of *Kermes vermilio* Planchon (*Hemiptera*: *Kermesidae*), a mediterranean pest of evergreen oaks. Brit J Entomol Nat Hist 21: 75-79.

Martín-Bernal E, Hernández-Alonso R, Ibarra N, Pérez-Fortea V, Cañada JF, 2002. Cochinillas de las encinas. *Kermes vermilio* Planch. Hemíptero. Fam. *Kermesidae.* In: La sanidad forestal en Aragón: 20 años de informaciones técnicas: 1985-2005. Gobierno de Aragón (Spain), Departamento de Medio Ambiente. pp: 157-160.

Martín M, Sánchez S, Batlle A, Laviña A, Barriuso J, 2012. First identification of the pathogen causing tumor malformations of evergreen oaks in Spain. In 1st International Congress of Trufficulture. 5-8 March, 2013. Teruel, Spain. Submitted to Forest Syst.

Mollá S, Villar P, Peñuelas JL, García-Fayos P, 2003. Influencia de la localización del vivero sobre la resistencia a la helada y el desarrollo en campo de *Quercus ilex* spp. *ballota* L. Montes 74: 23-30.

Moralejo E, García-Muñoz JA, Descals E, 2009. Susceptibility of Iberian trees of *Phytophthora ramorum* and

Phytophthora cinnamomi. Plant Pathol 58(2): 2071-283.

Nieves-Aldrey JL, 1998. Insectos que inducen la formación de agallas en las plantas: una fascinante interacción ecológica y evolutiva. Boletín sociedad entomología aragonesa. CSIC 23: 3-12.

Ono K, 1970. Effect of soil condition on the occurrence of Armillaria root rot of Japanese larch. Bull Govt Forest Exp. No, 299, Tokyo, Japan.

Pacioni G, Bologna MA, Laurenzi M, 1991. Insect attraction by *Tuber:* a chemical explanation. Mycol Res 95(12): 1359-1363.

Perez-Izquierdo L, Pulido F, 2013. Spatiotemporal variation in acorn production and damage in a Spanish holm oak (*Quercus ilex*) dehesa. Forest Syst 22(1): 106-113.

Pradel L, 1914. Manuel de Trufficulture. JB Baillière El fils, Paris, France.156 pp.

Reyna S, De Miguel AM, Palazón C, Honrubia M, Ágreda T, Águeda B, García-Barreda S, Martín-Santafé M, Moreno-Arroyo B, Oliach D, Sánchez S, Barriuso JJ, 2013. Spanish trufficulture. In 1st International Congress of Trufficulture. 5-8 March, 2013. Teruel, Spain.

Reyna S (ed). Truficultura. Fundamentos y Técnicas (2), 2012. Ed Mundi-Prensa, Madrid (Spain). 720 pp.

Reyna S, Gacia-Barreda S, Folch L, 2006. Influencia del uso del suelo sobre el potencial de inóculo ectomicorrícico y a competitividad de las ectomicorrizas de *Tuber melanosporum*: evaluacion mediante bioensayos en invernadero. Inv Agrar: Sist Rec For 15(3): 308-320.

Rivera CS, Blanco C, Oria R, Venturini ME, 2010. Diversity of culturable microorganisms and occurrence of *Listeria monocytogenes* and *Salmonella* spp. in *Tuber aestivum* and *Tuber melanosporum* ascocarps. Food Microbiol 27: 286-293.

Rubini A, Belfiori B, Passeri V, Baciarelli Falini L, Arcioni S, Riccioni C, Paolocci F, 2011. The AD-type ectomycorrhizas, one of the most common morphotypes present in truffle fields, result from fungi belonging to the *Trichophaea woolhopeia* species complex. Mycorrhiza 21: 17-25.

Sánchez ME, Caetano P, Ferraz J, Trapero A, 2002. *Phytophthora* disease of *Quercus ilex* in South-western Spain. Forest Pathol 32(1): 5-18.

Sánchez S, 2012. Ectomicorrizas en el cultivo de trufa negra: ecología, diversidad y gestión. Doctoral thesis. Universidad de Zaragoza, Zaragoza, Spain.

Sánchez S, Gómez E, Martín M, De Miguel AM, Urban A, Barriuso J, 2014. Experiments on the life cycle and factors affecting reproduction of *Sphaerosporella brunnea* provide evidence for rapid asexual propagation by conidiospores and for homothallism in an ectomycorrhizal competitor of cultivated truffle species. Fungal Ecology.

Schultz JC, Lechowicz MJ, 1986. Host plant, larval age and feeding behavior influence midgut pH in the gipsy moth (*Lymanrtia dispar*). Oecologia 71: 133-137.

Soria FJ, Cano E, Ocete ME, 1996. Efectos del ataque de fitófagos perforadores en el fruto de la encina (*Quercus rotundifolia* Lam.) Bol San Veg. Plagas 22: 427-432.

Soria S, López MM, Lopez-Lopez MJ, 1997. Presencia, sintomatología y daños de *Erwinia quercina* en España y su posible relación con la "seca de la encina". Ecología 11: 295-301.

Spodek M, Ben-Dov Y, 2012. Morphology of the first-instar nymph and adult female of *Kermes echinatus* Balachowsky, with a comparision to *K. vermilio* Planchon (Hemiptera, Coccoidea, Kermesidae). ZooKeys 246: 11-26.

Torres-Vila LM, Cruces E, Sánchez, A, Ferrero JJ, Ponce F, Martín D, Aza C, Rodríguez F, Barrena F, 2008. Dinámica poblacional y daños de *Curculio elephas* Gyllenhal (Col.: Curculionidae), *Cydia fagiglandana* Zeller, *Cydia triangulilla* Goeze y *Pammene fasciana* L. (Lep.: Tortricidar) sobre *Quercus* y *Castanea* en Extremadura. Bol San Veg. Plagas 34: 329-341.

Townes H, 1972. A light weight malaise trap. Entomological News 83: 239-247.

University of California, Agriculture & Natural Resources, 2013. UC-IPM [on line] http://www.ipm.ucdavis.edu/. [4 February 2014].

Zambonelli A, 1993. Effect of treatments against *Quercus* powdery mildew on the formation of mycorrhiza of *Tuber albidum* and *Tuber aestivum.* Informatore Fitopatologico 43(2): 59-62.