Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian Peninsula

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Abstract

In the last two centuries, several species of Australian eucalypts (e.g. *Eucalyptus camaldulensis* and *E. globulus*) were introduced into the Iberian Peninsula for the production of paper pulp. The effects of the introduction of exotic root-symbitotic fungi together with the eucalypts have received little attention. During the past years, we have investigated the biology of ectomycorrhizal fungi in eucalypt plantations in the Iberian Peninsula. In the plantations studied, we found fruit bodies of several Australian ectomycorrhizal fungi and identified their ectomycorrhizas with DNA molecular markers. The most frequent species were *Hydnangium carneum*, *Hymenogaster albus*, *Hysterangium inflatum*, *Labyrinthomyces donkii*, *Laccaria fraterna*, *Pisolithus albus*, *P. microcarpus*, *Rhulandiella berolinensis*, *Setchelliogaster rheophyllus*, and *Tricholoma eucalypticum*. These fungi were likely brought from Australia together with the eucalypts, and they seem to have facilitated the establishment of eucalypt plantations and their naturalization. The dispersion of Australian fungal propagules may be facilitating the spread of eucalypts along watercourses in semiarid regions increasing the water lost. Because ectomycorrhizal fungi are obligate symbionts, their capacity to persist after eradication of eucalypt stands, and/or to extend beyond forest plantations, would rely on the possibility to find compatible native host trees, and to outcompete the native ectomycorrhizal fungi. Here we illustrate the case of the Australasian species *Laccaria fraterna*, which fruits in Mediterranean shrublands of ectomycorrhizal species of *Cistus* (rockroses). We need to know which other Australasian fungi extend to the native ecosystems, if we are to predict environmental risks associated with the introduction of Australasian ectomycorrhizal fungi into the Iberian Peninsula.

Introduction

Alien plants often require mutualistic partners to overcome barriers to establishment in foreign environments. Mutualisms that facilitate invasions occur at several phases of the life cycle of alien invading plants. However, even when wind and native generalist animals mediate flower pollination and seed spread, the lack of compatible mycorrhizal symbionts can limit the spread of alien plants (Richardson et al. 2000a).

Mycorrhizal fungi are essential in plant nutrition in terrestrial ecosystems, and terrestrial plants present different types of obligate mycorrhizal symbioses. Herbs and shrubs form arbuscular mycorrhizas (AM) with glomales (Smith and Read 1997). The low specificity of AM relationships and the easy acquisition of mutualistic symbionts by herbs and shrubs in any ecosystems are important reasons for so many ecosystems being susceptible to invasion by alien plants. As a consequence, the introduction of AM fungi does not

seem to play a major role in mediating plant invasions, except on some remote islands that are poor in AM fungi (Richardson et al. 2000a). Most forest trees, however, associate with a group of basidiomycetes and ascomycetes forming ectomycorrhizas (ECM) (Newman and Reddell 1987). Ectomycorrhizal symbioses present a range of host–fungus specificities. For this reason, exotic forestry has often needed the introduction of compatible ectomycorrhizal fungal symbionts (Grove and Le Tacon 1993). For many non-native trees, notably for pines and eucalypts, the lack of symbionts was a major barrier to establishment and invasion in the southern hemisphere, before the build-up of inoculums through human activity (Armstrong and Hensbergen 1996; Davis et al. 1996; Richardson et al. 2000a). The role of ECM fungi in facilitating the establishment and invasion of alien trees was claimed by Richardson et al. (1994) to explain the invasion patterns of pines in South Africa. Little investigation, however, has been done on the role played by the ECM fungi in the naturalization of eucalypts beyond their natural range.

This paper provides a framework for thinking about the effects of the introduction of exotic fungi with plantations of exotic forest trees. Different sections will deal with aspects of the invasion biology of eucalypts and their ECM fungi. We first lay the groundwork for our paper by briefly introducing the history of the exotic forestry and several terms used in studies of biological invasions. The paper will be illustrated with a study conducted on exotic plantations of eucalypts in the Iberian Peninsula. We will describe ectomycorrhizal communities of Australasian fungi present in these plantations. We will next analyze the role that ECM fungi plays in promoting (or limiting) invasion rates of the eucalypts introduced. The central core of the paper will try to explain the lags between the introduction and the spread of the eucalypts, as a factor that depends on the dispersion of propagules of introduced ECM fungi. We report host shifts of Australian fungi to native ectomycorrhizal plants detected to date. We discuss whether the introduced fungi could threaten natural communities of ECM fungi by out-competing the native ECM fungi from their natural hosts. This work includes an analysis of the potential effects of the invasion of these exotic fungi in the nutrient cycling of Medi-

terranean forests. To the best of our knowledge, this is the first investigation dealing with the invasion ecology of exotic ECM fungi.

Exotic forestry: invasions of alien trees

Origin of exotic forestry

There is evidence of large-scale forestation in the ancient Mediterranean basin, where timber- and crop-producing trees were planted as long ago as 255 B.C. (Zobel et al. 1987). Inspite of its long history, the scale of forestry remained small until recently. Large-scale forestry was not widespread until the second half of the 20th century (Zobel et al. 1987), when pines and eucalypts were widely planted outside their natural ranges. Pines, which comprise only Holarctic species, were planted in South America, South Africa and Australia. The Australasian eucalypts were planted worldwide. In particular, the need for increased wood production to improve living conditions made the genus *Eucalyptus* one of the most widely planted silvicultural crops. In addition, in many damaged ecosystems, afforestation with alien eucalypts was driven by the belief that such plantings were beneficial to the environment (Zobel et al. 1987).

Naturalization and invasion of pines and eucalypts

In this paper, we will use the following three concepts: introduction, naturalization and invasion, as defined by Richardson et al. (2000b). A tree introduction takes place when humans transport a tree across a geographical barrier to a new area. Naturalization refers to the species establishing new self-perpetuating populations and becoming incorporated within the native flora. The naturalized trees regenerate freely, but mainly under their own canopies. In contrast, invasive species recruit seedlings, often in very large numbers, at long distances from parent plants (often more than 100 m). Only some of the naturalized plants become invasive, producing important environmental or economical damages.

All trees that are widely planted in alien environments can become invasive and spread under certain conditions (Richardson 1998). Consequently, the use of exotic trees has often caused

environmental damages in different parts of the world (Binggeli 1996). The species that cause the greatest problems are generally those planted most widely and for the longest time (Pryor 1991; Rejmánek and Richardson 1996). According to Higgins and Richardson (1998), at least 19 *Pinus* species are invaders of natural ecosystems in the southern hemisphere; four of the most widespread invasive pines are *P. halepensis*, *P. patula*, *P. pinaster* and *P. radiata* (Higgins and Richardson 1998; Richardson et al. 1990).

The genus *Eucalyptus* L'Hérit (Myrtaceae) comprises evergreen woody plants, including shrubs and forest trees (nearly 600 species), which are confined in natural occurrence entirely to the Australasian region, Papua, New Guinea and Timor (Pryor 1976). More than 43 species of eucalypt trees are planted outside their natural geographic distribution. Eucalypts are planted on a large scale to provide a short rotation crop yielding wood and paper pulp for industrial use (Eldridge et al. 1994). Although less invasive than pines, several species of eucalypts already caused problems as invaders in South Africa (Richardson 1998; Richardson et al. 2000a). Eucalypts are represented on many weed lists from other parts of the world, including California (Warner 1999) and peninsular Spain (Sanz-Elorza et al. 2001). Significant impacts might result from the introduction of eucalypts into Spain and some transformation on various ecosystem properties; specially changes in grasslands and scrubland habitats. Ecology and environmental politics dictate the desirability of maintaining Mediterranean grasslands and scrublands due to their high diversity in endemic plants and because these ecosystems are the natural habitats for many local wildlife. Eucalypt invasions can cause shifts in life-form dominance, reduced diversity, disruption of prevailing vegetation dynamics, and changing nutrient cycling patterns. Hence, eucalypt plantations are increasingly causing major conflicts between Spanish foresters, politicians and conservationists.

Role of ectomycorrhizal fungi in exotic forestry

Mycorrhizas of herbs, shrubs and forest trees

Mutualistic interactions between fungi and plant roots are common in the plant kingdom, including mycorrhizal symbioses. In most mycorrhizal symbioses, the fungal partner supplies nutrients to the host plant in exchange for photosynthetic carbon, and may offer protection against pathogens, toxins and drought (Smith and Read 1997). The main types of mycorrhizal symbioses differ in the anatomy of the mycorrhiza, which is a mixed root-fungus structure at which nutrient interchanges take place (Smith and Read 1997). Most Mediterranean plants are mycorrhizal with different types of fungi. Herbs and shrubs mainly associate with glomales to form arbuscular mycorrhizas (AM), which is by far the most common mycorrhizal symbiosis in the Mediterranean terrestrial ecosystems (Díez 1998). A restricted group of plants form particular types of mycorrhizas with a range of particular ascomycetes and basidiomycetes, such as the Ericales and Orchidales. However, most forest trees form ectomycorrhizas (EM) with a polyphyletic group of basidiomycetes and ascomycetes (Smith and Read 1997; Hibbett et al. 2000).

Forest trees are obligate ectomycorrhizal plants

Pines and eucalypts are obligate ectomycorrhizal trees, which depend on these mutalistic symbioses for nutrient uptake in natural conditions (Smith and Read 1997). Ectomycorrhizal symbioses are essential in the mobilization of nutrients in soil forests (Fahey 1992; Read and Pérez-Moreno 2003). In natural conditions, these forest trees rely on the ectomycorrhizal fungi, which colonize the root cortex and form a nutrient-gathering 'organ' called ectomycorrhiza (Smith and Read 1997). Ectomycorrhizas are almost exclusive to forest trees. In the Mediterranean, only a restricted group of shrubs in the Cistaceae (*Cistus* spp., called rockroses) forms EM, in which are involved a range of endemic ascomycetes and basidiomycetes (Díez 1998). Ectomycorrhizas are characterized structurally by the presence of a dense mass of fungal mycelium surrounding the short lateral roots (the mantle). The mantle originates from the attachment of the fungal hyphae onto epidermal cells, and the multiplication of hyphae to form a series of hyphal layers. The fungal mycelium also grows among the cortical cells, forming the Hartig net. The Hartig net is the structural and functional interface between fungal and roots cells. The fungal mantle is connected with a highly extended

network of mycelium prospecting the soil and gathering nutrients. The extra-radical mycelium is responsible for mobilizing soil nutrients, nutrient (and water) uptake and transfer to the ectomycorrhiza (Peterson and Bonfante 1994). Sclerotia (and sclerotia-like bodies) are vegetative balls of hyphae formed by a few species of ECM fungi. Finally, fruiting bodies arise from discrete points of the extra-radical mycelium to ensure the sexual reproduction and the dispersal of the fungal partner (Allen 1991).

The seedlings of forest trees need to be colonized by ectomycorrhizal fungi. In a given biotope, the seedling survival depends on the presence of the propagules of compatible ectomycorrhizal fungi in the soil (spores, sclerotia, or soil mycelium). Hyphae arising from fungal propagules have a limited capacity to grow and die unless they come in contact with a root tip of a compatible host. In the forest, seedlings grow near mycorrhizal trees and may thus become colonized by pre-existing mycorrhizal mycelia of the living roots of mature trees (Onguene and Kuyper 2002). In open areas, the fungal hyphae and then the sclerotia disappear in the long term in the absence of compatible ECM plants (Brundrett and Abbott 1995). Thus, the colonization of the root seedlings in a new biotope necessarily depends on the spore dispersion from close forests (Allen 1991). Spores of epigeous (fruiting aboveground) fungi are mainly dispersed by wind, whereas mycophagous animals are important vectors for dispersing spores of hypogeous (fruiting below ground) fungi.

Success of exotic forest plantations: the occurrence of compatible ectomycorrhizal fungi

In the AM symbiosis, the levels of specificity among host plants and fungal species are low, and many glomales have a cosmopolitan distribution (Smith and Read 1997). Due to this low specificity, most invading herbs and shrubs have no problems to form mycorrhizas with the fungi of the target habitat (Richardson et al. 2000a). In contrast, the ECM fungi present different levels of specificity. A given species of ectomycorrhizal fungus is usually only able to establish mutualistic symbiosis with a number of species from the same biogeographic realm, and even more highly specific interactions occur (Molina et al. 1992).

Specially, Australian ectomycorrhizal plants (e.g. *Eucalyptus*, *Acacias* spp.) have evolved in isolation from the ECM fungal flora associated with *Pinus* (and *Quercus)* in the northern hemisphere (Halling 2001). This could explain why many (if not almost all) native fungi from the northern hemisphere do not associate with eucalypts *in silva*, and *vice versa*.

When a compatible ectomycorrhizal biota is absent on the plantation site, there is a barrier for the success of exotic plantations. For this reason, mycorrhizal inoculation of pine and eucalypt seedlings with forest soil, spores or mycelium of compatible ECM fungi was often necessary for the success of exotic plantations of pines (Perry et al. 1987; Grove and Le Tacon 1993). In the Southern Hemisphere, pine forestry was delayed by the lack of suitable ECM fungi until a number of Holarctic ECM fungi were introduced with the pines (Dunstan et al. 1998). Some of these exotic pines eventually invaded a wide range of systems with the introduction of such Holartic ECM fungi (Richardson et al. 2000a).

Study areas and methods used to identify the ectomycorrhizal fungi

Plantations of eucalypts in Spain

Since the last century, the river red gum *Eucalyptus camaldulensis* Dehnh. and the blue gum *E. globulus* Labill. have been used in extensive plantations in the Iberian Peninsula. Nowadays, there are around 550,000 ha of eucalypts plantations in Spain, 320,000 ha of *E. globulus* and about 180,000 ha of *E. camaldulensis*. *Eucalyptus camaldulensis* is planted mainly in southwestern Spain (Huelva, Cadiz, Badajoz and Seville), and *E. globulus* in the northern regions (Galicia, Asturias and Santander). *Eucalyptus gomphocephala* DC is used on basic soils in Murcia and Almería (southeastern Spain). These eucalypts are used also in afforestation and agroforestry (windbreaks, shelter trees, and intercropping of trees and arable crops). In the Iberian Peninsula, the inoculation with ectomycorrhizal fungi was not necessary to ensure the success of many eucalypt plantations. Two hypotheses might account for the lack of need of inoculation: (i) whether there were native ectomycorrhizal fungal species

compatible with the eucalypts, (ii) or a range of ectomycorrhizal fungi native from Australasia were brought together with the eucalypt seedlings. To resolve this question, we have been investigating the origin of ectomycorrhizal fungi present in the Iberian plantations of eucalypts.

Study area and sampling strategy

The study area is located in the region of Extremadura, which is formed by the provinces of Cáceres and Badajoz, where extensive plantations with eucalypts took place between 1955 and 1977. *Eucalyptus camaldulensis* was by far the most predominant species, followed by *E. globulus*. Extremadura stands out with 14% of the totality of Spanish plantations of eucalypts. We studied eucalypts stands, shelterbelts and road verges. We sampled watercourses and riparian stands in which eucalypts became naturalized and invasive.

We sampled fruit bodies and ectomycorrhizal root tips. The fruit bodies are identified using morphological features. In some cases, the use of molecular and phylogenetic methods is necessary to discriminate among cryptic species (Díez et al. 2001). We collected ectomycorrhizal roots, because many ectomycorrhizal species do not fruit, and fruiting patterns do not truly reflect the belowground community of ECM fungi (Gardes and Bruns 1996; Horton and Bruns 2001).

Ectomycorrhiza identification: morphological and molecular methods

Despite the general organization, ectomycorrhizas differ among species in colour, mycelium density, size, forms and biochemical composition. Morphological typing of ectomycorrhizas enables us to identify fungi that seldom or never produce fruiting bodies. Methods for the morphological characterization of ectomycorrhizas are described in Ingleby et al. (1990) and Agerer (1997), who provided descriptions of ectomycorrhizas and criteria to discriminate species based on morphological features and chemical tests. Typing ectomycorrhizas with morphological methods is time consuming; and in many cases, it is not conclusive, because the ectomycorrhizas have not been described for many ECM fungi. In addition, the ectomycorrhizas of one fungal species can also show different morphologies according to the host, physiological and environmental conditions (Egger 1995). With such a morphological approach, we can classify the ectomycorrhizal tips as much as in morphotypes.

To overcome the problems of morphological typing, we use molecular methods for the identification of ectomycorrhizas, as described in Martín et al. (2000). Such methods are based on the restriction fragment length polymorphism (RFLP) of the internal transcribed sequences (ITS) of the nuclear rDNA. The ITS regions is amplified for ectomycorrhizal root tips with the polymerase reaction technique (PCR), using a thermostable DNA polymerase and primer pairs annealing at conserved regions of the 18S and 28S ribosomal genes (White et al. 1990). Such PCR-based methods are of great value in these kinds of studies (Glen et al. 2001a, b). We amplify the ITS regions from DNA obtained from ECM root tips, using fungal-specific primers to avoid the amplification of plant DNA (Gardes et al. 1991; Gardes and Bruns 1993). After cutting the PCR-amplified ITS with restriction enzymes, we obtain RFLP patterns.

To identify the different ectomycorrhizas, we are compiling a database of RFLP profiles of fruit bodies, so that we can compare them with those obtained from ECM roots. This PCR-RFLP database is of great help in the identification of unknown ectomycorrhizas. Most fungal species show a unique RFLP pattern, and their ectomycorrhizas can be identified by their PCR-RFLP profiles. For RFLP patterns not associated with any of the fruit bodies, direct sequencing of the ITS regions followed by a 'Blast research' (Altschul et al. 1997) in the National Centre for Biotechnology Information (http://www2.ncbi.nlm.nih.gov/) enable us to identify the ectomycorrhizal morphotypes, with luck, even at the species level.

Ectomycorrhizal fungi of eucalypt plantations in the Iberian Peninsula

Species of introduced Australian ectomycorrhizal fungi

Over our surveys in Iberian plantations of eucalypts, we found fungi known only from Australian forests and eucalypts plantations worldwide

Table 1. Twelve frequent Australasian fungi that have been introduced in the Iberian Peninsula together with the euca-lypts.

Taxonomic group	Species	Habit
Basidiomycetes	*Laccaria fraterna*[a]	Epigeous
	Hydnangium carneum[a]	Hypogeous
	Hymenogaster albus[a]	Hypogeous
	Hysterangium inflatum[a]	Hypogeous
	Pisolithus albus[a]	Epigeous
	Pisolithus microcarpus[a]	Epigeous
	Setchelliogaster rheophyllus[a]	Semi-hypogeous
	Tricholoma eucalypticum[a]	Epigeous
Ascomycetes	*Labyrinthomyces donkii*[a]	Hypogeous
	Rhulandiella berolinensis[a]	Hypogeous
	Discinella terrestris[c]	Epigeous
	Urnula rhytidia[b]	Epigeous

The table also includes a saprophytic fungus and a possible facultatively ectomycorrhizal fungus of Australasian origin.
[a] Ectomycorrhyzal.
[b] Saprophytic, and probably facultatively ectomycorrhizal.
[c] Saprophytic.

(Table 1). Our analyses of ECM roots confirmed the presence of Australian ECM fungi in the euca-lypt roots of Spanish plantations. Surveys of fruit body proved that fungi fruiting in the Iberian plantation of eucalypts are of Australian origin.

The most frequently occurring species in the Spanish plantations are *Hydnangium carneum* Wallr., *Hymenosgaster albus* (Klotzsch) Berkeley and Broome, *Hysterangium inflatum* Rod. (= *H. pterosporum* Donadini and Riousset), *Labyrinthomyces donkii* Malen ç. *Pisolithus albus* (Cke and Mass.) M.J. Priest, *P. microcarpus* (Cke and Mass.) Cunn., *Ruhlandiella berolinensis* (Henn.) Diss. and Korf, *Laccaria fraterna* (Cooke and Mass.) Sacc. (*L. lateritia* Malenç), *Setchelliogaster rheophyllus* (Ber. and Malenç.) G. Moreno and Kreisel, and *Tricholoma eucalypticum* Pearson. Most of these Australian fungi are able to fruit under the climatic conditions of the Iberian Peninsula. Additional ECM fungi, likely unable to fruit outside their natural range, were detected belowground during the molecular typing of ec-tomycorrhizal roots. We identified ectomycorrhi-zas of *Cenococcum geophilum* Fr. and several species of *Sebacina* and *Thelephora*. Whether the *C. geophilum*, *Sebacinia* and *Thelephora* strains infecting eucalypts in the Iberian plantations are natives or Australian deserves further studies, because these species are known to be native to the area studied.

In Extremadura, the soil dries up in summer, and most fungi in the eucalypt plantations fruit in late winter and spring. Some of these species are secotioids or truffle-like (sequestrate) fungi, most of them adapted to fruit belowground (hypogeous fungi). *Hydnangium carneum* is one of the most common truffle-like fungi in the eucalypt stands; which is a gastroid relative of the agaricoid genus *Laccaria;* due to its hypoge-ous habit, this Australian fungus is well adapted to the conditions of the Mediterranean climate. We also found the Australian false truffle *Hyme-nogaster albus,* which is common in plantations near the Monfragüe Natural Park. *Hysterangium inflatum* is hypogeous relative to *Phallus*, very common in the Monfragüe region. *Setchelliogas-ter rheophyllus* is another secotioid fungus pres-ent in eucalypt plantations near Monfragüe and Badajoz. *Rhulandiella berolinensis* is considered specific to Australasian ectomycorrhizal plants, and is very common in riparian stands of *Euca-lyptus camaldulensis* near Mérida (Badajoz). The main epigeous fungus (fruiting aboveground) was *Laccaria fraterna*, which is an agaricoid species native to Australia and introduced into the Medi-terranean with eucalypts. To the best of our knowledge, our collections of *Tricholoma euca-lypticum* are among the first records of this fun-gus outside Australia.

Growing on debris of eucalypts and humus of eucalypt plantations, we found the Australasian species *Urnula rhytidia* (Berk.) Cooke. (Pezizales) and *Discinella terrestris* (Berk. and Br.) Dennis (Leotiales); these two species are regarded as characteristic of sclerophyllous eucalypt forests of Australia and Tasmania. There is no reliable information on whether these fungi are sapro-phytic or facultatively ectomycorrhizal as many other pezizales.

In the studied plantations of eucalypts in Ex-tremadura, we found strains of the Australasian species *Pisolithus albus* and *P. microcarpus*, as proved with molecular analyses of the ITS sequences of the nuclear rDNA (Díez et al. 2001). For many years, the name *Pisolithus ar-rhizus* (Pers.) Rauscher (synonym of *P. tinctorius* [Pers.] Coker and Couch) have been used for all *Pisolithus* strains occurring in eucalypt and pine plantations worldwide, regardless of the host plant (Cairney 2002). This misunderstanding occurred because many researchers considered

Pisolithus as a monospecific genus, and *P. arhizus* as a fungus with a wide host range (Chambers and Cairney 1999). However, our molecular analyses proved that the genus *Pisolithus* comprises several phylogenetic species, and that each species of *Pisolithus* is confined to hosts from one single biogeographic realm. Our study also proved that *Pisolithus arhizus* is restricted to Holarctic host plants (e.g. *Quercus* and *Pinus* spp.) and does not occur in eucalypt plantations (Martin et al. 2002). In a previous work (Díez et al. 2001), we showed that *Pisolithus albus* and *P. microcarpus* fruit in litter and on open ground and at the edges of eucalyptus plantations, and on dry and disturbed sites such as gravelly roadsides in Morocco and Spain. Endemic to Australia, *P. albus* and *P. microparpus* are not restricted to eucalypts and form ectomycorrhizas with other Australasian plants; we have found these two species in association with Australasian acacias in Portugal (Muriel and Díez, unpublished). The species of *Pisolithus* native to the Iberian Peninsula correspond to *P. tinctorius*, and two unnamed species, one basophilic species and another *Cistus*-specific *Pisolithus* species (Díez et al. 2001); these three Holarctic species of *Pisolithus* never occur in association with eucalypts (Díez et al. 2001; Martin et al. 2002).

A group of Australasian ectomycorrhizal fungi were introduced with the eucalypts

The fungi we found in the Iberian plantations of eucalypts are of Australian origin. These exotic fungi were likely introduced with eucalypt seedlings brought into peninsular Spain before plant quarantine restrictions were observed. In Australia, gum seedlings are container grown in nurseries, which are naturally colonized by a limited number of ectomycorrhizal fungi. Most of these ECM fungal species may persist during the first years of eucalypt plantations (Lu et al. 1999). Foresters probably dispersed these exotic ECM fungi in soil or eucalypt seedlings worldwide. Our results are in agreement with investigations by other mycologists, and there is a growing consensus in a worldwide dispersal of a number of Australasian ECM fungi together with the eucalypts (Giachini et al. 2000). Saprophytic and even pathogenic fungi seem to have spread worldwide as well with the eucalypts. In this regard, it has been suggested that dissemination of the basidiomycetous yeast *Cryptococcus neoformans*, a human pathogen associated with *Eucalyptus* leaves in southern California and India resulted from the introduction of eucalypts (Casadevall and Perfect 1998; Chakrabarti et al. 1997).

Do native fungi infect exotic eucalypts in sylva?

In the Iberian Peninsula, there are many native ECM fungi in association with pines, oaks, and a restricted number of ectomycorrhizal shrubs (i.e. *Cistus* spp.) (Díez 1998). In our surveys, we did not find Holarctic ECM fungi in the eucalypt plantations, though propagules of native fungi are often present on planting sites. We do not know any reliable evidence of European fungi forming ectomycorrhizas with eucalypts in natural conditions in Spain. Because eucalypts have evolved in isolation from the ectomycorrhizal mycobiota associated with *Pinus* and *Quercus* in the Holarctic Realm, the native fungi (e.g. *Pisolithus tinctorius*) might be unable to associate with eucalypts *in sylva*. Even within each biogeographic realm, there would exist some level of host-symbiont specificity. Parladé et al. (1996) described, in pure culture syntheses, the ability of native Iberian fungi to colonize several North American conifers planted in northern Spain. Such an ability can be easily explained by the similarities between the fungal floras of North America and the Iberian Peninsula (Halling 2001), as these two regions belong to the Holarctic Realm. However, some host specificity might exist, because in tree nurseries and experimental plantations in northern Spain, native ECM fungi do not seem to outcompete exotic North American fungi inoculated (or that accidentally infect seedlings) in tree nurseries (Pera et al. 1999).

Role of the introduction of Australasian ectomycorrhizal fungi in promoting eucalypt invasiveness

We now have evidence on the introduction of Australian ectomycorrhizal fungi with the plantations of eucalypts in the Iberian Peninsula. Such introductions seem to mediate the naturalization of eucalypts, because once dense tree plantations are established, new seedlings can become

ectomycorrhizal very rapidly through infection from the established fungal network. In many cases, the introduced eucalypts compete with native species, and the eradication of the eucalypts is difficult, especially in areas with a long history of large and extensive plantations. In Spain, the regional and national governments are promoting the eradication of eucalypts from natural and national parks.

An example is the National Park of Doñana. Doñana is well known for its variety of species of birds, either permanent residents, winter visitors from north and central Europe, or summer visitors from Africa, such as numerous types of geese and colourful colonies of flamingo. Doñana's configuration is the result of its past as the estuary of the Guadalquivir river, and mainly consists of beaches, coastal mobile dunes, marshes and lakes. Fauna in Doñana is rich and some in danger of extinction, such as the Iberian lynx, the Egyptian mongoose and the imperial eagle. The local wildlife of Doñana depends on the water level. Autumn rains brought life back to the marshes and filled the lagoons after the dry summer. Gradually, the water attains a uniform depth of 30–60 cm over vast areas, and the resulting marshes attract flocks of water birds of the most varied kind. Among other causes, the level of the freshwater of the marshes is in danger as a result of intensive plantations of eucalypts (Sacks et al. 1992). In the Doñana National Park (southern Spain), the eradication of eucalypts is necessary to reduce the water loss and the conservation of the local wildlife (e.g. Iberian lynx, *Felix pardina*) (Palomares et al. 1991).

The eucalypts introduced are competitive with native species, and their control and eradication are difficult. These eucalypts and the associated exotic ECM fungi can regenerate from root fragments. We know as well that ectomycorrhizal spores can remain dormant in soil for long periods, and might colonize eucalypt seedlings grown from remaining eucalypt seeds Moreover, ectomycorrhizal eucalypt seedlings often efficiently compete for soil nutrients with the planted young trees of native *Quercus* species (i.e. *Q. ilex* L., *Q. pyrenaica* Willd. and *Q. suber* L) (Muriel and Díez, unpublished). These reasons would account for the naturalization of eucalypts in Peninsular Spain (Vila et al. 2001) and in other regions of the Mediterranean Basin (Le Floc'h 1991).

In the checklist of alien species in Spain, Sanz-Elorza et al. (2001) classified the eucalypts as alien plants with a clear invasive behaviour, and as dangerous (causing ecological damage and alterations) for natural ecosystems. To date, the invasion behaviour is limited to areas with a long history of large and extensive plantations. In Spain, the red river gum often invades along the watercourse, which is its natural habitat in Australasia. In these areas, the plantations of eucalyptus have a great impact on lowering water tables, and have a devastating ecological impact, reducing soil quality and the habitat of local wildlife. The invasive tree species have a predictable set of life-history attributes, including low seed mass and short juvenile periods, and a short interval between seed crops (Rejmánek and Rhichardson 1996). Many eucalypts present many of these characteristics and produce large quantities of small seeds easily propagated by wind or/and animals (Pryor 1976). In addition, the pollination and seed dispersal do not limit the eucalypt invasiveness. Most eucalypts species are facultative outbreeders and are pollinated by a variety of generalist insects, in natural forest and exotic plantations (Pryor 1976). Therefore, the limited success of eucalypts as efficient invaders in the Iberian Peninsula can be puzzling. One can expect eucalypts to be successful as invaders, because these species are likely to be different in their resource utilization, easily escaping competition with native species (Richardson 1998).

To date, the difficulties of the eucalypts to find compatible fungal partners within the Iberian fungal biota seem to restrict their spread in the Iberian Peninsula to areas close to large plantations. The expansion of the invasion of the eucalypts from the plantation sites is likely to be hindered by the lack of compatible ectomycorrhizal fungi at potential seedling recruitment places. Lack of, or low extent of, colonization by compatible ectomycorrhizal fungi may be an important factor preventing or reducing seedling establishment of these alien trees. Eucalypt invasions would often result from the dispersal and propagules of Australian fungi. The importance of eucalypts as invaders correlates with the extent and the duration of planting, which relates to the dispersal rates of ECM propagules. The spread of propagules will facilitate a successful seedling establishment in new biotopes. As stressed above,

the spread of ECM fungi in new biotopes is often by air spores, which is slow (Brundrett and Abbott 1995). This could explain why eucalypts might exist in plantations for many years, before they start to invade indigenous vegetation. A factor contributing to this lag is that compatible ectomycorrhizal propagules, in the form of spores (often air-born spores), needed time to accumulate in the soil (spore bank) before eucalypts can establish and proliferate. However, it is only a matter of time before the fungal spore bank reaches sufficiently high levels to allow ectomycorrhizal eucalypts to spread everywhere in compatible environments.

Invasiveness of Australasian ectomycorrhizal fungi

Awareness by politicians of the negative effects of exotic trees on natural biodiversity has led to an attempt to eradicate eucalypts in natural parks in Spain. But do Australasian ectomycorrhizal fungi persist after eucalypt eradication? After the removal of eucalypts, there will persist a high level of inoculums (spores, mycelium) of Australasian ectomycorrhizal fungi, which might colonize the roots of native trees that are planted on old eucalypt plantation sites. We have little information of the ability of the introduced Australian fungi to infect plants native to the Iberian Peninsula. It would be necessary to investigate whether these Australian fungi colonize roots of the Mediterranean trees planted on former eucalypt plantations. We do not know whether these exotic fungi spread beyond the plantations and colonize native ECM flora.

ECM fungi need to live in association with the tree roots, and their spread beyond the plantations will depend on (i) their compatibility with any native ectomycorrhizal plants, and (ii) their ability to exclude the indigenous ECM fungi. In our survey, we did not find Australian fungi in association with native ectomycorrhizal plants, except *Laccaria fraterna*, which naturally occurs in association with eucalypts in Australia and worldwide plantations. We found this Australian fungus in shrublands of the ectomycorrhizal shrub *Cistus ladanifer* L. near plantations of eucalypts. In two sites near the Monfragüe Natural Park, fruiting bodies of *L. fraterna* were found 500 and 700 m far from the nearest eucalypt tree,

and no eucalypt seedlings were found near the fruiting bodies of *L. fraterna*. Molecular typing of the ectomycorrhizal root tips identified *L. fraterna* on roots of *Cistus ladanifer* (Muriel and Diez, unpublished). We do not know whether these native woody plants that are associated with exotic ectomycorrhizal fungi (i.e. *C. ladanifer*) have access to resources that these native plants normally cannot tap, which would modify the nutrient cycling and affect the ecosystem functioning.

In our study, we detected the Australian fungi *Urnula rhytidia* and *Discinella terrestis,* which are considered as saprophytic (or facultatively ectomycorrhizal). These fungi should have come with the eucalypts. *Urnula rhytidia* was also found on fallen leaves of *Quercus ilex* L., in oak woodlands near eucalypt plantations in Badajoz. These data suggest that many other saprophytic fungi could be introduced with the eucalypts and might spread to native ecosystems. Due to their saprophytic life style, these exotic fungi might persist for a long time after the eradication of the eucalypts, altering nutrient cycling in the soil.

Limiting further introduction of exotic EM

Australian forests have one of the most privative and rich mycobiota of the world (Castellano and Bougher 1994; Bougher and Syme 1998), but only a few Australian ECM fungi seem to have been introduced into the Iberian Peninsula. The limited functional and genetic diversity of introduced ECM species should be determining the environment range that eucalypts are able to invade. However, we continue to move soil and microbes around the world to establish new plantations, which favours the introduction of more and more Australian ECM fungi. Particular ECM fungal species might confer unique advantages for obtaining nutrients in potential habitats or to use particular nutrient resources (Buscot et al. 2000). An increased diversity of introduced Australian ECM fungi might increase the ability of the eucalypts to invade new habitats in the Iberian Peninsula.

Some authors propose to increase the diversity of the introduced ECM fungi to improve the productivity of exotic eucalypt plantations (Dell et al. 2002). This may involve selecting hardy

ECM strains tolerating a wide range of edaphic and environmental conditions and strains that elicit growth responses of eucalypt plantations (Neves-Machado 1995). Among these inoculums, there may be highly competitive strains, which could make the natural ecosystems more vulnerable to invasion by eucalypts. This would include strains coping with extremely harsh or toxic soil conditions. Rare and ecologically sensitive ecosystems, such as serpentine communities, may be particularly vulnerable to the introduction of Australasian ECM fungi with broad environmental tolerances.

Elevated levels of atmospheric carbon dioxide (global climate change) create an increasing concern. Exotic plantations are now promoted for their presumed capacity to provide a net sink of atmospheric carbon, and mycorrhizal fungi may play a critical role in terrestrial carbon exchange processes (Staddon et al. 2002). Chapela et al. (2001) described how exotic ectomycorrhizal fungi induce soil carbon depletion in pine plantations in Central America. We have no information on the impact the Australasian fungi could have on the cycles of nutrients in the Iberian soils.

Several methods for the transformation of ectomycorrhizal fungi are already available (Hanif et al. 2002; Pardo et al. 2002). Some scientists propose to use strains genetically manipulated to form better symbiotic systems, including ECM fungi which are more efficient in mobilizing nutrients from soils. This might result in eucalypt species that do not invade at present but become invasive if associated with such selected (or genetically modified) fungal strains.

Conclusions

Several conclusions can be drawn from our studies on the ectomycorrhizas of eucalypt plantations in the Iberian Peninsula:

(1) In the Iberian Peninsula, a number of Australasian ECM fungi were introduced together with the eucalypts. The reduced number of Australian ECM fungal species, together with the low ability of Iberian fungi to colonize eucalypt roots, would explain the low diversity of ECM fungal communities in exotic stands of eucalypts.

(2) The introduction of these Australasian ECM fungi appears to be one of the main factors accounting for the successful establishment of eucalypt plantations in the Iberian Peninsula, their naturalization, and invasive behaviour. Consequently, a deeper knowledge on the ectomycorrhizas of eucalypt stands in the Iberian plantations will help to refine our ability to predict the invasiveness of the eucalypts introduced. The knowledge generated may be crucial for determining potential endangerment and to suggest strategies for protecting the diversity of the Mediterranean ecosystems.

(3) It will be necessary to investigate potential host shifts of Australian fungi to native hosts, and their effects on the native ECM fungal communities and on the functioning of Mediterranean ecosystems. For these reasons, it is urgent to investigate the ectomycorrhizas of native trees planted in former plantation sites, and roots of indigenous ectomycorrhizal plants growing near eucalypt plantations.

(4) The present investigation highlights the need to regulate the translocation of ectomycorrhizal fungi for forest inoculations. Quarantine measures would be necessary to control any future introduction of ECM fungi of Australian origin.

(5) Before introducing beneficial Australasian strains of ECM fungi, we would recommend screening their ability to improve eucalypt invasiveness and to infect roots of native ECM plants. Screening the invasiveness of introduced strains will help to prevent negative effects on Iberian natural ecosystems.

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