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Mycorrhizal synthesis of *Lactarius indigo* (Schw.) Fr. with five Neotropical pine species

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Abstract This paper describes for the first time the ectomycorrhiza synthesized between two Guatemalan strains of *Lactarius indigo* (Schw.) Fr. and the Neotropical species *Pinus ayacahuite* var. *ayacahuite* Ehren, *P. hartwegii* Lindl., *P. oocarpa* Schiede ex Schltdl. var *oocarpa*, *P. pseudostrobus* Lindl. and *P. rudis* Endl. The synthesis was carried out in a controlled growth chamber using plastic containers with peat moss-vermiculite substrate and mycelial inoculum. Mycorrhiza were obtained 25 days after inoculation. A description of the morphology, appearance and structure of mantle and Hartig net is given for each combination. Mycorrhiza were saffron to cinnamon greenish with age, with a net of saffron laticifers visible through outer mantle; orange latex secreted when injured. Cystidia-like emanating hyphae were observed on the mantle surface of young mycorrhiza. Plectenchymatous mantle with abundant interhyphal gelatinous material.

Keywords *Lactarius indigo* · Ectomycorrhiza · Neotropical pines · Cystidia · Mycelial inoculation

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Introduction

Lactarius indigo (Schw.) Fr. (blue-milk cap mushroom) is a very common edible fungus with a wide distribution, from East Asia (China and Japan) to northeast America (Hesler and Smith 1979; Hutchinson 1991) and in Central America including Colombia, with a disjunct distribution (Mueller and Halling 1995; Wu and Mueller 1997; Halling 2001).

This species is associated with pines or oaks in North America (Hesler and Smith 1979), but in Mexico, it has been found to be associated to *Alnus jorulensis* H.B.K., *Carpinus carolineana* Walt., *Ostrya virginiana* (Mill) K. Kotch. and *Liquidambar macrophylla* Oersted (Montoya and Bandala 1996). In Guatemala, this fungal species has been reported in association with *Pinus pseudostrobus* Lindl., *Pinus* spp. and *Quercus* spp. (Morales et al. 2002), *Pinus rudis* Endl. (Flores et al. 2002a) and *P. caribaea* Morelet (Flores and Bran, unpublished data), and recently, it was found in a mixed forest of pines, oaks and *Liquidambar stiraciflua* L. (Morales, personal communication). In Costa Rica and Colombia, it is found only under *Quercus* (Mueller and Halling 1995). This change of host plant may be the result of migration and adaptation to new environments, as was suggested by Singer (1988) and Wu and Mueller (1997).

As for other species of the section Dapetes, *L. indigo* is highly appreciated as an edible mushroom, whose harvest time depends on the season and the latitude of the countries where it grows. In Mexico, fruit bodies are collected from June to November and sold under several names in local markets (Montoya and Bandala 1996). In Guatemala, *L. indigo* is sold from May to October together with *Lactarius deliciosus* s.l. and with other edible mushrooms (Flores et al. 2002b).

Despite its frequency in its natural habitat and the use of fruit bodies as food, *L. indigo* mycorrhiza have received little attention. Only one attempt to obtain mycorrhiza by inoculating agar-mycelial inoculum of *L. indigo* on *Pinus strobus* seedlings has been reported, but it failed (Marx and Kenney 1982).

Furthermore, the knowledge of the mycorrhizal status of the Neotropical pines *Pinus ayacahuite* Ehrenb. Ex Schltdl. *P. hartwegii* Lindl., *P. oocarpa* Schiede ex Schltdl., *P. pseudostrobus* and *P. rudis* is very scarce, and only a few putative associations have been reported (Arias and Garza 1999; Donahue et al. 1991; Flores et al. 2002a; Flores 2003). *P. pseudostrobus* and *P. oocarpa* are two widely distributed pine species in Mesoamerica, the latter representing about 50% of the pine forests in Guatemala and 90% in Nicaragua. In contrast, *P. ayacahuite* presents a reduced population from South Mexico to Honduras (Veblen 1978), where it may grow between 1,800- and 3,200-m altitude. *P. hartwegii* and *P. rudis* also have a scarce distribution in Mexico and Guatemala, growing between 3,000- and 3,700- and 2,200- to 3,300-m altitude, respectively. In these high environments, the establishment of mycorrhizal symbiosis may be critical due to climatic and nutritional stress (Haselwandter 1987). The taxonomic position of *P. hartwegii* and *P. rudis* has been widely discussed. Until recently, they were considered as different species due to phenotypical and ecological characteristics (Perry 1991). However, Matos (1995) proposed them as synonyms on the basis of molecular analysis.

The aim of the study described in this paper was to obtain the mycorrhizal synthesis between the appreciated edible fungus *L. indigo* and some Neotropical pine species. This is the first step for the potential establishment of a protocol for controlled mycorrhization with this economically valuable fungus. Taking into account the increasing interest of reforestation programmes in Central America, the use of edible mushrooms such as *L. indigo* could be an important economic diversification in these areas. The mycorrhiza of *L. indigo* have not been previously described. Morphoanatomical analysis of the mycorrhiza could be a useful tool for assessing the inoculation success and detecting fungal contaminations in nurseries, so this study was also aimed at morphotyping and characterizing the *L. indigo* mycorrhiza. Special attention was focused on distinguishing features of the mycorrhiza among the pine species.

Materials and methods

Plant material

Certified seeds of *P. ayacahuite* var. *ayacahuite*, *P. oocarpa* var. *oocarpa* and *P. pseudostrobus* were obtained from the Instituto Nacional de Bosques de Guatemala (INAB). *P. hartwegii* and *P. rudis* seeds were collected in Tuicoj, Todos Santos Cuchumatán, northwest Guatemala, in 2000–2001.

The seeds were disinfected for 30 min in 30% H_2O_2, rinsed in sterile distilled water, seeded in plastic trays with sterilized vermiculite (120°C×1 h) for germination and placed in a growth chamber (19–23°C, 65% RH and 16:8-h light/dark cycle with 2,500 lx as maximum illumination). Distilled sterile water was used for humidification of the vermiculite until germination. Once germinated, the seedlings were transferred to 300-ml containers previously filled with sterilized (120°C, 1 h, three times on alternate days) peat moss/vermiculite (1:1, v/v) and maintained in the growth chamber for 6 months (with the same conditions but light intensity increased to 12,500 lx) until short root development. Plants were watered and fertilized (Peter's Professional 20-7-19 NPK) when needed.

Fungal strains and inoculation procedures

Three Guatemalan strains of *L. indigo* collected by R. Flores were used in the experiment (Table 1). Voucher specimens have been deposited at the "Rubén Mayorga Peralta" Herbarium, at the University of San Carlos, Guatemala. They were isolated in BAF medium (Moser 1960) by explants from carpophore tissue and later cultivated in flasks with semiliquid BAF medium (1.5 g agar/l) in the dark at 23°C. In order to increase fungal biomass production, previously determined optimal pH values (Flores 2003) were used: pH 4.5 for *L. indigo* SM58.00 and pH 5.5 for *L. indigo* USAC 8.00 and *L. indigo* EN126.00. Prior to inoculation, the mycelia were filtered through a 65-μm net and washed in sterile water in order to eliminate sugars and to reduce the growth of saprophytic contaminants in the seedling root systems. To assure mycorrhizal synthesis, the seedlings were inoculated twice. The first inoculation was made with mycelium fragmented and homogenized by manual agitation and then resuspended in distilled water (35–40 g of mycelium in 500 ml of water), applying 30 ml of the mycelial suspension to the seedling rootlets. A second inoculation was made 1 month later with mycelial pellets, obtained by mechanical agitation of mycelia with a shaker (GFL), placing 20–40 pellets on the rootlets.

Table 1 Origin, associated plant species and date of collection of the *Lactarius indigo* strains

Strain	Origin	Associated plant species	Date of collection
L. indigo USAC 8.00	San Carlos University Campus, Guatemala; 1,500-m altitude	*Pinus oocarpa*	06/2000
L. indigo SM 58.00	San Mateo Ixtatán, Huehuetenango, Guatemala; 3,600-m altitude	*Pinus rudis*, *P. hartwegii*	06/2000
L. indigo EN 126.00	Finca El Naranjo, Guatemala City, Guatemala, 1,600-m altitude	*Pinus pseudostrobus*, *P. oocarpa*, *Quercus peduncularis*	08/2000

Voucher specimens deposited at the herbarium of University of Murcia (MUB)

Mycorrhiza descriptions

Mycorrhiza were described following Agerer (1987–1996) and Ingleby et al. (1990). Fresh mycorrhiza were described and photographed using an Olympus SZH zoom stereomicroscope. The mycorrhiza were fixed in formol–alcohol–acetic acid (FAA), washed in distilled water and put into an ultrasonic cleaner (Fungilab S.A) to detach substrate debris from the surfaces. Samples selected for semithin sections were washed three times in cold phosphate buffer, 0.2 M, pH 7.2, included in 1% OsO4 for 2.30 h and maintained for 2 h in uracile acetate. Serial ethanol dehydration and Spurr resin embedding were carried out according to Hall and Hawes (1991).

One-micrometre sections were made with a Reichert Jung Ultracut microtome and using crystal knives. Sections were stained with 1% toluidine blue in 1% acetic acid and then observed and photographed using a light Olympus BH-2 microscope.

Mycorrhiza for scanning electron microscopy (SEM) were cleaned and washed as described above and prepared according to Hall and Hawes (1991). Gold-coated samples were observed in a Jeol T-300 scanning electron microscope, and photographs were saved by Autobeam Link Issis Program. Voucher samples of the fixed mycorrhiza are deposited at the Laboratory of Mycology-Mycorrhiza of the University of Murcia.

Results

Mycorrhiza of *L. indigo* were obtained 20–25 days after pellet inoculation. Both SM58.00 and USAC 8.00 strains were effective in producing mycorrhiza, but not the strain EN126.00.

P. ayacahuite+*L. indigo* SM58.00

Morphological characters Mycorrhizal system fairly short, stiped, dichotomous, up to 2.2 mm long (Fig. 1a, Fig. 2a, b). *Main axis* up to 1.8 mm length and (0.4) 0.45–0.50 mm in

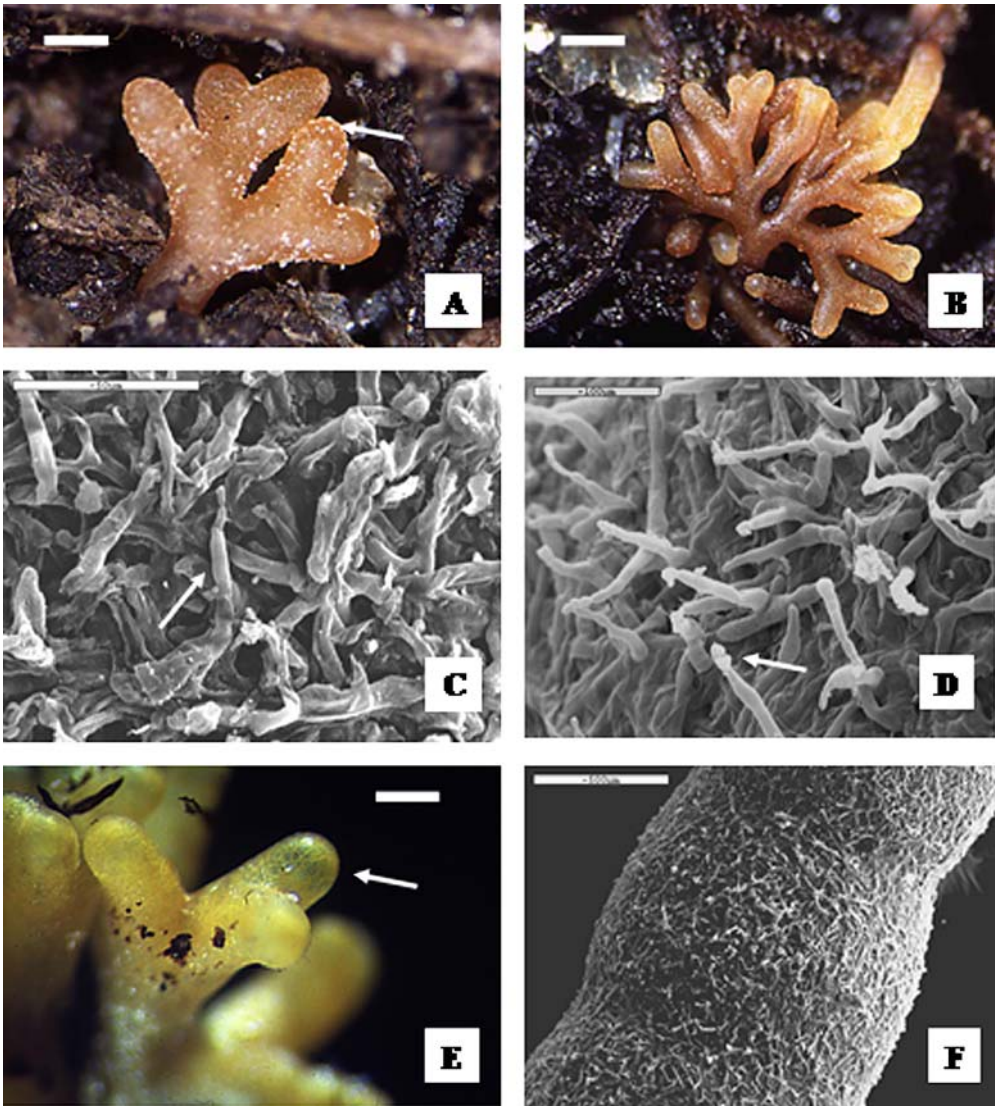

Fig. 1 *L. indigo* ectomycorrhiza. **a** On *P. ayacahuite*, coarse granulations. **b** On *P. rudis*, reticular and grainy surface. **c** On *P. oocarpa*, cystidia-like hyphae with beaded apex. **d** On *P. pseudostrobus*, cystidia-like hyphae in young mycorrhiza. **e** On *P. oocarpa*, yellow-orange laticifers below the surface of young mycorrhiza. **f** On *P. oocarpa*, surface of young mycorrhiza with abundant cystidia-like hyphae. **a**, **e** *Bar*=0.4 mm; **b** *bar*=1.25 mm; **c** *bar*=50 μm; **d** *bar*=100 μm; **f** *bar*=500 μm

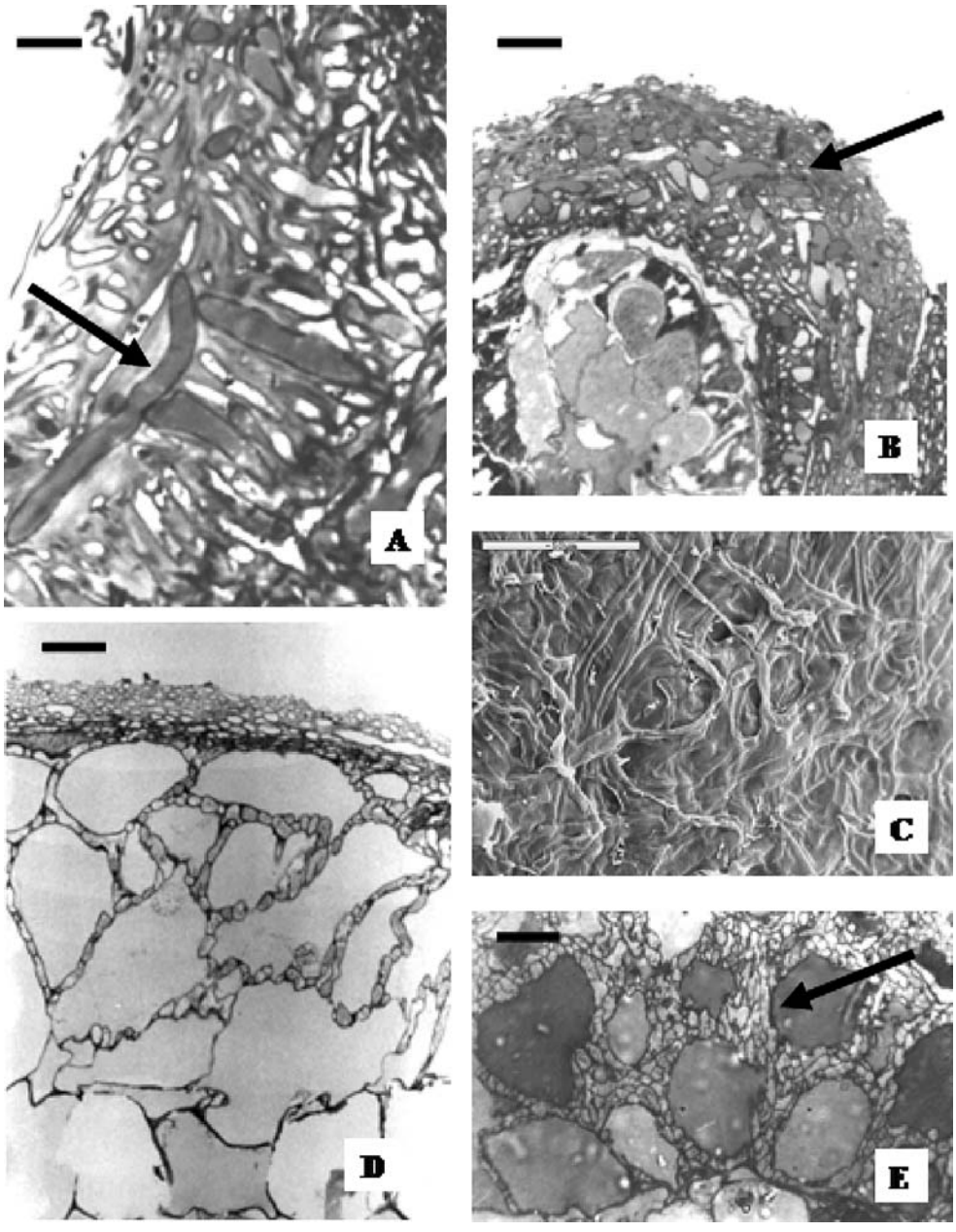

Fig. 2 *L. indigo* ectomycorrhiza. **a** On *P. ayacahuite*, thick laticifers surrounded by hyphae and gelatinous material (tangential section through mantle). **b** On *P. ayacahuite*, cross-section showing thick mantle with laticifers. **c** On *P. pseudostrobus*, plectenchymatous mantle showing hyphae and gelatinous material (surface views of ECM, SEM). **d** On *P. oocarpa*, cross-section with mantle and Hartig net. **e** On *P. pseudostrobus*, cross-section showing thick Hartig net. **a** *Bar*=10 μm; **b**, **d**, **e** *bars*=20 μm; **c** *bar*=50 μm

diameter. *Unramified ends* rather straight, up to 0.8 mm in length and (0.40) 0.45–0.48 mm in diameter, yellowish in young mycorrhiza to saffron at maturity, net of saffron laticifers visible through outer mantle, apices lighter, yellowish to saffron, older parts of ectomycorrhiza become rusty tawny to cinnamon with dark green spots, whole mycorrhiza turning cinnamon greenish with age; mantle and rhizomorphs secrete orange milk when injured. *Surface of unramified ends* short spiny (acute cystidia-like emanating hyphae, up to 31 μm long) in young ectomycorrhiza, especially at the tips but reticulated and grainy (whitish crystalline grains) and pruinose base in older ones. *Rhizomorphs* rare, emanating from the basal part of the ectomycorrhiza, up to 0.7 mm in diameter, roundish to flattened in cross-section, surface reticulated with dispersed short spiny hyphae up to 23 μm length; young rhizomorphs thin and transparent, older ones thicker and coloured like the mantle, turning green with age or damage.

Anatomical characters of mantle in cross-section Mantle 12–42 μm thick, densely plectenchymatous, with abundant interhyphal gelatinous matrix material, two to three layers distinguishable, outermost layer compact, laticifers frequent in the middle and inner layers. All hyphae without clamps. *Outermost mantle layer* hyphae usually fasciculate to dispersed in a thick gelatinous matrix; hyphae rather straight, 3–4 μm in diameter, branched; laticifers rare, 4–6 μm in diameter. *Middle mantle layer* hyphae more agglutinated than those of the outer mantle layer, no pattern discernable; hyphae 3–4 μm in diameter, laticifers frequent,

6–10 μm in diameter, frequently branched. *Innermost mantle layer* hyphae tightly agglutinated by interhyphal matrix; hyphae 3–4 μm in diameter, laticifers frequent, 6–10 μm in diameter.

Hartig net surrounds two to three of the four to five rows of cortical cells with a single chain of irregular hyphae, 3.0–6.0 μm in diameter and 4.0 to 18.0 μm length. Tannin cells are surrounded by polygonal and irregular hyphae, 2.0–4.0 μm in diameter and 4.0–10.0 μm in length.

P. rudis+*L. indigo* SM58.00

Morphological characters Mycorrhizal systems fairly short, up to 3.8 mm long, dichotomous, stiped, forming clusters (Fig. 1b). *Main axis* 0.5–0.55 mm in diameter and up to 2.2 mm long. *Unramified ends* rather straight, up to 0.9-mm length and 0.5–0.55 mm diameter; yellowish in young mycorrhiza but saffron at maturity; net of saffron coloured laticifers visible through the outer mantle; apices lighter to saffron or greenish; older parts of ectomycorrhiza cinnamon to dark green; whole mycorrhiza turning brown-dark green with age; mantle and rhizomorphs secrete orange latex when injured. *Surface of unramified ends* short spiny (acute cystidia-like emanating hyphae, up to 32 μm long) especially at the tips of young mycorrhiza but reticular to grainy (whitish crystal-like grains) in older ones; bases smooth to finely fibrillose. *Rhizomorphs* quite rare, emanating from the basal part of the ectomycorrhiza, up to 0.6 mm in diameter, roundish to flattened in cross-section, surface rugulose-reticulate to finely grainy (transparent granules) with monopodial ramification; young rhizomorphs thin and transparent, older ones thicker and coloured like the mantle, turning green with age or when injured.

Anatomical characters of mantle in longitudinal section Mantle very thin 12–20 μm thick, plectenchymatous, with abundant interhyphal gelatinous material, two layers distinguishable, outermost layer compact, especially towards the apex, laticifers frequent in the innermost layer. All hyphae without clamps. *Outermost mantle layer* with large areas of gelatinous material, hyphae dispersed, rather straight, 3–4 μm in diameter; laticifers rare, 4–6 μm in diameter, ramified and septate. *Innermost mantle layer* hyphae 3–4 μm in diameter, tightly agglutinated by interhyphal gelatinous matrix, laticifers frequent, 5–6 μm in diameter, ramified and septate.

Hartig net scarce, surrounds one to two of the three rows of cortical cells with a single chain of irregular hyphae, 3–8 μm in diameter and 4–14 μm in length. Tannin cells are surrounded by irregular hyphae 3–6 μm in diameter and 4–12 μm in length.

P. hartwegii+*L. indigo* SM58.00

Morphological characters Mycorrhizal systems fairly short, stiped, dichotomous, up to 4.0 mm, often forming clusters. *Main axis* 0.40–0.45 mm in diameter and up to 2.0 mm long. *Unramified ends* rather straight, 0.35–4.0 mm in diameter, yellowish in young mycorrhiza to saffron at maturity; net of saffron coloured laticifers visible through outer mantle; apices sometimes beaded, lighter, yellowish to saffron, older parts of ectomycorrhiza cinnamon to dark green; whole mycorrhiza turning brownish with age; mantle and thicker rhizomorphs secrete orange latex when injured. *Surface of unramified ends* short spiny (beaded cystidia-like emanating hyphae, up to 47 μm long) in young ectomycorrhiza, especially at the tips, but reticulated to grainy (whitish crystalline-like granules up to 31 μm in diameter) especially at the basal and middle parts of the mycorrhiza. *Rhizomorphs* not found.

Anatomical characters of mantle in longitudinal section Mantle thin, 14–30 μm thick, densely plectenchymatous, with abundant interhyphal gelatinous material, two layers distinguishable, especially towards apex; outer layer compact; all hyphae without clamps. *Outermost mantle layer* hyphae dispersed to tightly adjoined in the gelatinous matrix, hyphal cells rather straight, frequently branched, 3–4 μm in diameter; laticifers rare, 4–6 μm in diameter. *Innermost mantle layer* Hyphae tightly agglutinated by the interhyphal gelatinous matrix, hyphae 3–4 μm in diameter, forming layers; laticifers scarce, septate, 3–4 μm in diameter.

Hartig net, when present, reaches the endodermis with a single chain of irregular and elongate hyphae, 3–5 μm in diameter and 8–12 μm long. Tannin cells are surrounded by irregular hyphae of 3–6 μm in diameter and 4–12 μm in length.

P. oocarpa var. *oocarpa*+*L. indigo* USAC 8.00

Morphological characters Mycorrhizal systems fairly short, stiped, dichotomous, up to 4.5 mm long. *Main axis* up to 2.0 mm long and (0.35) 0.40–0.45 (0.50) mm in diameter (Fig. 1c,e,f, Fig. 2d). *Unramified ends* rather straight, up to 0.6 mm long and 0.4–0.5 mm in diameter, yellowish in young mycorrhiza but saffron-peach at maturity; net of laticifers visible through outer mantle, apices lighter to orange or with green spots, older parts of ectomycorrhiza rusty tawny to cinnamon with green spots, whole mycorrhiza turning greenish brown with age; mantle secrete orange milk when injured. *Surface of unramified ends* short spiny (acute cystidia-like emanating hyphae, up to 29 μm long) especially in young ectomycorrhiza and pruinose to finely grainy (whitish grains) in mature mycorrhiza; surface of mycorrhizal bases very pruinose. *Rhizomorphs* emanating from the middle and basal part of the ectomycorrhiza, up to 86 μm in diameter, roundish to flattened in cross-section, surface rugulose with disperse short spiny hyphae (up to 31 μm long); young rhizomorphs thin and yellowish, older ones thicker and coloured like the mantle, turning greenish with age or when injured.

Anatomical characters of mantle in longitudinal section Mantle 16–40 (50) μm thick, densely plectenchymatous,

with abundant interhyphal gelatinous matrix material, two to three different layers distinguishable, laticifers usually frequent in the middle and innermost layers. All hyphae without clamps. *Outermost mantle layer* compact and thicker, with abundant emanating cystidia-like hyphae on the surface, hyphae in the gelatinous matrix tightly adjoining to dispersed; hyphal cells rather straight, 3–4 μm in diameter, frequently branched; some laticifers 3–5 μm are present. *Middle mantle layer* hyphae 3–4 μm in diameter, tightly united without any precise distribution, laticifers very frequent, branched, up to 5–7 μm in diameter. *Innermost layer* hyphae 3–4 μm in diameter, tightly agglutinated by the interhyphal matrix material; laticifers frequent, 4–8 μm in diameter.

Hartig net surrounds two to three of the three to four rows of cortical cells with one chain of irregular hyphae, 3–6 μm in diameter and 6–16 μm long. Sometimes, first rows can be surrounded by two or more chains of irregular hyphae. Tannin cells surrounded by thicker and irregular hyphae, 5–8 μm in diameter and 8–12 μm long.

P. pseudostrobus+*L. indigo* USAC 8.00

Morphological characters Mycorrhizal systems dichotomous, stiped, fairly short, up to 4.0 mm long, often forming clusters (Fig. 1d, Fig. 2c,e). *Main axis* 0.5–0.6 mm in diameter and 1.2 mm long. *Surface of ramified ends* rather straight, up to 0.9 mm long and 0.3–0.4 mm in diameter, yellowish in young mycorrhiza to saffron-peach at maturity, net of orange laticifers visible through outer mantle; apices lighter saffron, older parts of ectomycorrhiza rusty tawny to cinnamon, whole mycorrhiza turning greenish brown with age; mantle and rhizomorphs secrete orange milk when injured. *Surface of unramified ends* short spiny (beaded cystidia-like emanating hyphae, up to 31 μm long) in young ectomycorrhiza but reticulated to grainy (whitish crystal-like granules) in older mycorrhiza; bases of older mycorrhiza finely fibrillose to grainy. *Rhizomorphs* emanating from the basal part of the ectomycorrhiza, up to 62 μm in diameter, roundish to flattened in cross-section, surface rugulose with dispersed hyphae what cause a short-spiny appearance of the rhizomorphs; young rhizomorphs thin and yellowish, older ones thicker and coloured like the mantle, orange to greenish.

Anatomical characters of mantle in cross-section Mantle 14–30 μm thick, densely plectenchymatous, with abundant interhyphal gelatinous matrix material, two to three different layers distinguishable, outermost layer compact, laticifers present but more frequent in middle and innermost layers. All hyphae without clamps. *Outermost mantle layer* hyphae in the gelatinous matrix tightly arranged or dispersed, rather straight, 3–4 μm in diameter, branched and septate; laticifers rare, 3–4 μm in diameter. *Middle mantle layers* hyphae more agglutinated than in the outer layer, 3–4 μm in diameter, laticifers frequent, 5–7 μm in diameter. *Innermost layer* hyphae tightly arranged and agglutinated in the matrix material, septate, 3–4 μm in diameter, laticifers frequent, ramified, 4–6 μm in diameter. *Very tips* show thinner mantle with less matrix material.

Hartig net surrounds three of the four rows of cortical cells with a single chain of irregular hyphae, 4–6 μm in diameter and 4–12 μm long. Tannin cells surrounded by irregular hyphae, 4 μm in diameter and 6–8 μm long.

Note: with the *L. indigo* SM58.00 strain, ectomycorrhiza were quite similar but sometimes the mantle was thicker, up to 80 μm thick, especially in the innermost layer; laticifers 3–5 μm in diameter and ramified; hyphae 3–4 μm in diameter in both layers; Hartig net also reached the third cortical row but with several chains of irregular hyphae around the cells.

Discussion

The mycorrhiza obtained between *L. indigo* and the six Neotropical pines species used had similar colours and morphological characteristics. However, the mycorrhiza synthetized in *P. rudis* and partially in *P. hartwegii* showed a thinner mantle than the others. Currently, both pines are considered as the same species (Matos 1995), a fact that would explain the anatomical similarity of both mycorrhiza. Although *L. indigo* has never been reported in association with *P. ayacahuite* in natural Guatemalan ecosystems, we obtained successful mycorrhizal synthesis and suggest the possibility to find ectomycorrhiza and fungal fruit bodies associated with *P. ayacahuite* in areas where this pine grows close to mixed with *P. rudis* and *P. hartwegii*. Important features of these mycorrhizal combination are the thick mantle and the presence of wide and frequent laticifers. *P. oocarpa* and *P. pseudostrobus* are common species in the Guatemalan highlands and are frequent partners in this specific symbiosis. In this study, we observed mantle thickness up to 80 μm, larger than the 15–35 μm reported for the genus *Lactarius* (Hutchinson, 1999).

The *L. indigo* mycorrhiza described here agrees in general with the description of *Lactarius*-type mycorrhiza pointed by Hutchinson (1999), in particular, in the plectenchymatous organization of the mantle found in several species and the presence of laticiferous hyphae in the inner layer of the mantle. These anatomical features are similar to those in other *Lactarius* species of the Section *Dapetes*, such as *L. deliciosus* (L.:Fr.) SF Gray (Torres and Honrubia 1994; García 1998; Guerin-Laguette 1998; Rincón 1998; Kernaghan et al. 1997), *L. salmonicolor* Herm & Leclair (Pillukat 1996; Comandini et al. 1998; Eberhardt et al. 2000), *L. sanguifluus* (Paulet ex Fr.) Fr. (Guerin-Laguette 1998; García 1998; Rincón 1998) and *L. deterrimus* Gröger (Agerer 1986). Some similarities were also found with species of other sections, as *L. porninsis* Rolland, Section *Zonari* (Treu 1990), and *L. intermedius* (Fr.) Cooke, Section *Tricholomoidei* (Eberhardt et al. 2000).

One important distinguishing feature of the *L. indigo* mycorrhiza is the presence of a short spiny surface in young ectomycorrhiza and a grainy surface in older ones. We have found emanating cystidia-like hyphae, with acute

or beaded ends and 31–47 μm in length, especially at the unramified ends. These hyphae disappear totally or partially as the mycorrhiza mature. Although the presence of cystidia is a frequent feature of the mycorrhiza formed by other closely related ectomycorrhizal fungi, such as *Russula* species (Kernaghan et al. 1997; Brand 1991), this kind of hyphae or cystidia in the genus *Lactarius* has only been reported for *L. rubrocinctus* (Brand 1991), *L. scrobiculatus* (Amiet and Egli 1991), *L. mitissimus* (Weiss 1991), *L. acris* (Brand 1992) and *L. lignyotus* (Kraigher et al. 1995). The presence of crystals was also previously observed by Eberhardt et al. (2000) on the surface of *L. intermedius* mycorrhiza with *Abies alba* and also by Weiss (1991) on the mycorrhiza of *L. mitissimus* with *Picea abies*.

Another interesting aspect of the *L. indigo* mycorrhiza is its orange to greenish colour and the presence of watery orange latex in the mycorrhizal mantle and rhizomorphs, which contrasts with the characteristic blue latex in the fruit bodies. Similarity in colour between fruit bodies and mycorrhizal mantle seems to be a consistent feature in *Lactarius* mycorrhiza. Indeed, the presence of laticiferous concolourous with latex of sporocarps is described for other *Lactarius* mycorrhiza of the section Dapetes (Pillukat, 1996). In this sense, it is interesting to note that the blue colour of the latex of *L. indigo* is only present in fruit bodies and in the aerial mycelium of cultures. However, submerged mycelia in semiliquid or solid medium are saffron to green or dark green in colours. In flasks with peat moss/vermiculite, the mycelium and rhizomorphs were also saffron to greenish.

The mycorrhizal synthesis obtained in this study demonstrated the mycorrhizal ability of the edible *L. indigo* to form symbiotic relationship with a range of host pine species. This fact could be helpful for the practical use of *L. indigo* as an edible mycorrhizal mushroom for reforestation programmes in Latin America, especially in Central America.

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