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TYPE OF LIGHT AND FORMATION OF BASIDIOMATA OF TWO SPECIES OF EDIBLE ECTOMYCORRHIZAL MUSHROOMS ASSOCIATED WITH NEO-TROPICAL PINES AND THE DESCRIPTION OF BASIDIOMATA DEVELOPMENT

TIPO DE LUZ Y FORMACIÓN DE BASIDIOMAS DE DOS ESPECIES COMESTIBLES DE HONGOS ECTOMICORRÍCICOS ASOCIADOS CON PINOS NEOTROPICALES Y DESCRIPCIÓN DEL DESARROLLO DE BASIDIOMAS

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SUMMARY

Ectomycorrhiza is a mutualistic symbiosis of paramount importance in the functioning of forest ecosystems. Therefore, inoculation with ectomycorrhizal fungi, particularly edible species, has gained enormous relevance in the production of forest trees with economic importance. However, due to technical difficulties for the cultivation of ectomycorrhizal basidiomata associated with their host trees, factors influencing basidiomata formation have not been fully understood. This study evaluated the effect of two light wavelengths on the production of basidiomata of two edible ectomycorrhizal mushroom species (*Hebeloma leucosarx* and *Laccaria bicolor*) associated with two neo-tropical trees, *Pinus montezumae* and *Pinus greggii*. The two fungal species evaluated are widely used as food and sold in traditional markets in México. In general, the type of light differentially influenced the basidioma formation of these fungi in association with host seedlings in greenhouse during six years. Increased basidioma formation of *H. leucosarx* was observed associated with trees growing in pots covered with yellow filters with a wavelength of ~590 nm compared with those covered with red filters with a wavelength of ~660 nm. Meanwhile, the opposite trend was observed in basidiomata of *L. bicolor*. The host tree species influenced the formation of basidiomata. *Hebeloma leucosarx* produced more basidiomata in association with *P. montezumae* than with *P. greggii*. In addition, a description of basidiomata formation from very young stages previous to senescence is provided for both mycobionts. To our knowledge, this is the first record of the effect of light wavelengths on the production of basidiomata of edible ectomycorrhizal mushrooms, demonstrating that this phenomenon is more complex than previously believed.

Index words: Ectomycorrhizal symbiosis, edible wild mushrooms, fertile basidiomata formation, red light, yellow light.

RESUMEN

La Ectomicorriza es una simbiosis mutualista de importancia primordial para el funcionamiento de los ecosistemas forestales. Por tanto, la inoculación con hongos ectomicorrícicos, particularmente especies comestibles, ha adquirido enorme relevancia en la producción de árboles forestales de importancia económica. Sin embargo, debido a las dificultades técnicas para el cultivo de basidiomas ectomicorrícicos asociados a sus árboles hospederos, los factores que influyen en la formación de basidiomas no han logrado ser completamente comprendidos. Este estudio evaluó el efecto de dos longitudes de onda de luz en la producción de basidiomas de dos especies de setas ectomicorrícicas comestibles (*Hebeloma leucosarx* y *Laccaria bicolor*) asociadas con dos especies de árboles neo-tropicales, *Pinus montezumae* y *Pinus greggii*. Las dos especies fúngicas evaluadas son ampliamente utilizadas como alimento y se venden en mercados tradicionales en México. En general, el tipo de luz influenció diferencialmente la formación de basidiomas de estos hongos en asociación con plántulas hospederas en invernadero crecidas durante seis años. La formación del basidioma del *H. leucosarx* se observó asociada a los árboles que crecían en las macetas cubiertas con filtros amarillos de una longitud de onda de ~ 590 nm, comparadas con las cubiertas con filtros rojos de una longitud de onda de ~ 660 nm. En cambio, la tendencia opuesta fue observada en basidiomas de *L. bicolor*. La especie del árbol huésped influenció la formación de basidiomas. *Hebeloma leucosarx* produjo más basidiomas cuando estuvo en en asociación con *P. montezumae* que en asociación con *P. greggii*. Aquí también se aporta una descripción de la formación del basidiomas de etapas muy jóvenes previas a la senescencia, para ambos micobiontes. Hasta donde se pudo verificar, este es el primer registro del efecto de las longitudes de onda de luz en la producción de basidiomas de setas ectomicorrícicas comestibles, lo que demuestra que este fenómeno es más complejo de lo que se creía.

Palabras clave: Simbiosis ectomicorrízica, hongos silvestres comestibles, formación de basidiomas fértiles, luz azul, luz amarilla.

INTRODUCTION

The ectomycorrhiza is a mutualistic symbiosis of enormous functional importance in boreal, temperate and some subtropical and tropical ecosystems (Pérez-Moreno and Read, 2001a, b; Read and Pérez-Moreno, 2003). Today, the use of ectomycorrhizal fungi, particularly of edible mushroom species, has gained immense importance in forest tree production (Smith and Read, 2010). Species of edible ectomycorrhizal mushrooms in *Hebeloma* and *Laccaria* are found worldwide (Cairney and Chambers, 1999). The worldwide numbers of recognized species for these edible ectomycorrhizal genera are 75 species for *Laccaria* (Mueller, 1985), and 150 and 124 species for *Hebeloma*, according to Kirk *et al.* (2008) and Index Fungorum (2017), respectively.

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The generic name of *Hebeloma* comes from the ancient Greek word *Hebe*, meaning 'youth' or 'puberty,' and the suffix - *loma*, meaning 'a fringe' or 'youthful veil,' in reference to the fungal veil that is only seen in immature stages of basidiomata of this genus. Both genera have great potential for use in nursery-based plant inoculation programs because they include pioneer species that thrive in low fertility conditions and that are associated with a wide range of host trees (Obase *et al.*, 2009; Trocha *et al.*, 2007), including species of great forest importance in the genera *Eucalyptus, Larix, Picea, Pinus* and *Pseudotsuga* (Danielson, 1984; Baar *et al.*, 1994; Wong *et al.*, 1989).

Several studies have reported on the basidiomata production of edible ectomycorrhizal mushrooms, such as *L. laccata* (Scop) Cooke, *H. cylindrosporum* Romagn. and *H. sarcophyllum* (Peck) Sacc. (Debaud and Gay, 1987; Wang and Hall, 2004). Additionally, some other species of edible ectomycorrhizal mushrooms have sporadically produced basidiomata under controlled conditions; for example, *Cantharellus cibarius* Fr., *Lactarius deliciosus* (L.) Gray, *Tricholoma portentosum* (Fr) Quél and *Rhizopogon roseolus* (Corda) Th. Fr. (Danell and Camacho, 1997; Danell, 2002; Guerin-Laguette *et al.*, 2000; Yamada *et al.*, 2001).

It has previously been reported that the factors influencing basidiomata formation in edible ectomycorrhizal mushrooms associated with their plant hosts are mainly temperature, fertilization regime (Debaud and Gay, 1987; Ellis *et al.*, 1999), relative humidity (Yamada *et al.*, 2007), the contribution of host carbohydrates and even electrical impulses (Ohga *et al.*, 2001).

It has also been reported that light regulates the fungal development and behavior of some molds and yeasts that belong to the Ascomycota. For example, a blue light-activated complex has been identified in *Neurospora crassa* as the product of the WC-1 and WC-2 genes (Linden *et al.*, 1997); and the hyphal cell growth of *Schizosaccharomyces japonicus* is a response to the light which is dependent on the WC-1 and WC-2 orthologs (Okamato *et al.*, 2013). Additionally, previous studies showed the importance of some wavelengths in the cultivation of saprobic Basidiomycetes including *Pleurotus tuber-regium, P. ostreatus, Hypsizygus marmoreus*, and *Lentinula edodes* (Jang *et al.*, 2013; Kuforiji and Fasidi, 2009; Nakazawa *et al.*, 2008). However, to our knowledge, the influence of light wavelength on basidiomata formation in edible ectomycorrhizal mushrooms has not been reported yet.

The aim of this study was to evaluate the effect of two light filters, yellow and red, on basidiomata formation in two edible ectomycorrhizal mushrooms, *L. bicolor* (Maire) P. D. Orton and *H. leucosarx* P. D. Orton, associated with two neo-tropical pines of great economic importance: *P. greggii* Engelm. ex Parl. and *P. montezumae* Lamb. In addition, the full developmental process of these basidiomata, undescribed so far, from mycelial aggregates of a primordium to hymenophore maturation of a matured basidioma is reported and illustrated in detail.

MATERIALS AND METHODS

Vegetative and fungal material and plant inoculation

Basidiomata of *Laccaria* and *Hebeloma* were obtained in a market in Ozumba, State of México, México, and identified according to macro and microscopic features, following conventional methods (Largent, 1973; Mueller, 1992). Species obtained included *L. laccata, L. bicolor, L. proxima* (Bound) Pat., *H. mesophaeum* (Pers.) Quél., *H. alpinum* (J. Favre) Bruchet and *H. leucosarx* P.D. Orton. Of these species, *L. bicolor* and *H. leucosarx* were selected for this study because they have a great economic and cultural importance in central México, and also have a great potential in the production of inoculum for trees of two neotropical species native to México, *P. greggii* (Domínguez *et al.*, 2001) and *P. montezumae* (Calderón *et al.*, 2006). Both pines are of great forest importance and used in reforestation in degraded sites due to their high survival percentage and economic importance as timbers in México and Central America.

Seeds of both pines came from natural forests in Xochicoatlán, Hidalgo and Cofre de Perote, Veracruz. Before planting, the seeds were sterilized with hydrogen peroxide (H_2O_2) at 30 % for 20 min and rinsed three times with sterile distilled water; the 140 cm^3 black plastic cylindrical containers (Merck, Baltimore, EUA) were also cleaned and disinfected with 70 % ethanol prior to being used. The substrate was added to 90 % of the total volume of the containers, and then spore inoculum was applied in a homogeneous layer.

Then, the seeds of the species were placed according to the treatments, just after the spore addition, and they were covered with a surface layer of the same substrate, and finally, a layer of "tezontle" (ground volcanic rock) was added. The substrate used consisted of a mixture of sand, pine bark and forest soil at a 2:2:1 ratio; which was sterilized in an autoclave (Aesa CV-250, Puebla, México) at 103 kPa and a temperature of 125 ºC for 5 h. One hundred and twenty mL of this substrate were added to each container.

The pilei of *L. bicolor* and *H. leucosarx* were cut from their stipes, since pilei contain spores in their hymenophores. These pilei were dehydrated in an oven at 35 ºC for 48 h, then ground in a mill (Thomas Wiley, Minnesota, USA) and finally sieved in a 1.19-mm-opening mesh to homogenize the size of the particles obtained. The inoculum was kept in 1.5-mL Eppendorf plastic vials at a temperature of 5 °C until use. Each plant was inoculated with 10^7 to 10^8 spores, which were counted in a Neubauer chamber (Marienfeld, Lauda-Konigshofen, Germany).

After a year, the plants were transferred to 1000 cm^3 pots containing the same substrate and remained there in the greenhouse for the next four years, being watered every other day with sterile distilled water. *P. greggii* and *P. montezumae* trees with a mycorrhizal colonization percentage of 90 % or more, were selected for transplantation into pots with a capacity of 7200 cm^3 (Venecia, Chicoloapan, México), containing the same substrate. To evaluate the ectomycorrhizal colonization, a modification of the method proposed by Grand and Harvey (1982) was used. In this case, three 98 cm^3-cylindrical (20 x 1.25 cm, height and radium, respectively) soil cores were extracted from the pots where the trees were grown for four years, by using PVC tubes; and the percentage of colonization was evaluated in them.

Experimental design and statistical analysis

The experimental design used was completely randomized with eight treatments. Two pine species, *P. greggii* and *P. montezumae*, were inoculated with each of *L. bicolor* or *H. leucosarx*, separately. The soil surface of the 7200 cm^3 pots used for each of the plant fungus combinations was covered with a yellow paper filter (Manila paper, Lumen, Toluca, México) with a wavelength of ~ 590 nm, or a red one with a wavelength of ~ 660 nm, leaving the above-ground part uncovered. For each of the 8 treatments thus generated, there were four replicates, giving a total of 32 pots, each one constituted by a tree. Treatments were arranged in a randomized experimental design. Data analyses for the number of basidiomata produced per pot consisted in the estimation of 95 % confidence limits (Snedecor and Cochran, 1989) for each treatment using Statgraphic Plus 5.1.

Experimental setup and evaluation of variables

The five-year old inoculated *P. greggii* and *P. montezumae* trees, covered with the two filter colors, remained for an additional period of a year in the greenhouse in the 7200 cm^3 containers and were irrigated every other day. Assessments were made of the basidiomata formed during four months (from October 2013 to January 2014), because their formation was only detected at this time of the year. The age of the trees at the end of the experiment was thus 6 years old. A total number of basidiomata formed in each treatment was counted twice a week, and a detailed description was made of the basidioma development of *L. bicolor* and *H. leucosarx*, from the mycelial aggregates of a juvenile primordium to the fertile mature basidiomata (Table 1). Photographs of the developmental stages of the basidiomata were taken with an Olympus SZ61, SZ2-LGB model stereoscopic microscope (USA). Glass slides were placed under those basidiomata to collect basidiospores, to record the color of spore print and to evaluate the spore micromorphology and size. The morphology and size of the spores were evaluated with a LEICA MD1000 model microscope (Germany).

RESULTS

Frequency of basidiomata formation

A total of 102 mature basidiomata (each with a fully developed hymenophore) of *L. bicolor* and *H. leucosarx* was recorded. Ninety percent of them corresponded to *H. leucosarx* and 10 % to *L. bicolor*. Fifty-five basidiomata were associated with *P. montezumae* and 47 were associated with *P. greggii.* Basidiomata formation was recorded from 280 to 330 d after transplantation into the 7200 cm^3 pots. The first basidioma formation in *H. leucosarx* was observed associated with *P. montezumae*.

Effect of light on basidiomata formation

There was a conspicuous effect of two light filters on the basidiomata formation of *Laccaria* and *Hebeloma*. A greater number of *H. leucosarx* than *L. bicolor* basidiomata, was observed regardless of the host plant. The number of *Hebeloma* basidiomata was 2.6 and 2.1 times higher when pot soil was exposed to the yellow filter as opposed to the red filter, in *P. montezumae* and *P. greggii*, respectively. The number of *L. bicolor* basidiomata recorded was relatively small: eight and one basidiomata in association with *P. greggii* exposed to red filter and yellow filter, respectively. No basidiomata of *L. bicolor* were observed in association with *P. montezumae* (Figure 1). The longevity of *L. bicolor* basidiomata, from a small visible primordium until senescent basidiomata, averaged 18 d. By contrast, the time from the mycelial aggregates to the senescence of *H. leucosarx* basidiomata averaged 37 d. In the case of *L. bicolor*, abundant whitish spore print was observed on glass slides 14 d after the appearance of visible primordia.

Developmental pattern of *H. leucosarx* and *L. bicolor* basidiomata

H. leucosarx. At 280 d after transplantation into the 7200 cm^3 pots, white mycelial aggregates were observed on the soil surface with *P. montezumae* (Figures 2c, 2e). After this initial phase, the small, white to whitish primordia (Figure

Table 1. Description of the developmental pattern of *Laccaria bicolor* and *Hebeloma leucosarx* basidiomata, formed in association with six-year-old *Pinus greggii* and *P. montezumae* seedlings.

Stage		*Laccaria bicolor* (Figure 3)	*Hebeloma leucosarx* (Figure 2)
I	Mycelial aggregate	Cluster of irregularly shaped mycelium, whitish or cream-colored, cottony, around 500 µm in size (Figure 3a).	Cluster of irregularly-shaped mycelium, whitish or cream-colored, cottony to velvety, around 10 mm in size (Figure 2e).
II	Primordium	Semispherical (Figure 3b), pyriform structure (Figure 3c), later oblong to sub-cylindrical, cottony or fibrous, velvety, purple at the base and whitish at the top, sometimes primordia can be completely whitish for short periods of time and then purple again, with a length of 1 to 4 mm (Figure 3d).	Hemispheric to convex or bell-shaped structure, the center reddish brown and the edge light brown to whitish, with a length of 4 mm (Figure 2f).
III	Immature basidiomata	Structure with a conspicuous stipe, with deep violet base and clear purple to whitish middle and upper part, clear formation of a pileus, inconspicuous to initially umbonate and subsequently flattened, about 3cm in length (Figure 3e); and finally subspherical, 4cm in diameter (Fig 3f).	Structure with a hemispherical (Figure 2g) to convex pileus (Figure 2h), whitish to light brown on the edge, reddish in the middle, stipe completely covered with longitudinal, creamy white fibrils, about 8cm.
IV	Mature basidiomata	Basidioma with a convex to flat pileus, finely fibrous or scaly with gills present, deep purple stipe at the base and lighter on top, about 7cm in height, white to whitish spore prints (Figure 3g).	Basidioma with a convex to umbonate pileus, light brown on the edge, reddish in the central disk, gills more or less separated, slightly dark brown (Figure 2i), stipe cylindrical to sub-cylindrical, up to 20 cm in length, thin and light dark brown spore prints.
V	Senescent basidiomata	Basidoma where the pileus and stipe loses their deep purple tone, becoming dark brown without turgidity, pileus of about 3cm in diameter (Figure 3h).	Basidioma where the pileus and stipe lose their light cream-colored tone, the central disk acquires a dark black tone, without turgidity (Figure 2j), pileus of around 6cm and stipe of 4cm in diameter.

2f), began to widen at their stipe base while the pileus began to form immature basidiomata (Figures 2f and 2g). The stipe secondarily elongated (Figure 2h). The cream-colored gills of the young basidiomata turned light brown as the pileus matured and expanded symmetrically (Figures 2h and 2i).

The shape of the pileus ranged from subglobose to hemispherical, later convex and flat in fully mature basidiomata (Figures 2i and 2j). Spores (Figure 2k) were almond-shaped [9.36-12.05 (Lm = 10.95 µm) x 5.27-7.39 (Wm = 6.225 µm) µm and Q = 1.63-1.77 (Qm = 1.76)] where Q means the ratio of length/width for all spores measured. This is the first description of the developmental pattern of basidiomata of *H. leucosarx.* In general, there is little information on *H. leucosarx,* as most *Hebeloma* articles focus on *H. crustuliniforme* and *H. cylindrosporum* (Cairney and Chambers, 1999).

L. bicolor. At 280 d after transplantation, white mycelial aggregates were observed at the stem base of *P. greggii* trees (Figure 3a). After this initial phase, the small primordia (Figures 3b and 3d), began to widen at their stipe base and developed violet-colored young basidiomata (Figure 3e). Matured basidiomata showed subglobose pileus (Figure 3f), later convex and finally flat (Figure 3g), finely fibrous or scaly, with pink gills (Figure 3f). The stipe's base was deep violet but its top displayed a lighter color. Spore print was white to whitish, spores were globose, and spiny ornamentation was observed (Figure 3h). The above-described features of *L. bicolor* match the description given for field basidiomata by Mueller (1992). The basidiomata formation was recorded along with the ectomycorrhizal structures, the host roots, including mantle, Hartig net and external hyphae (Figure 3i).

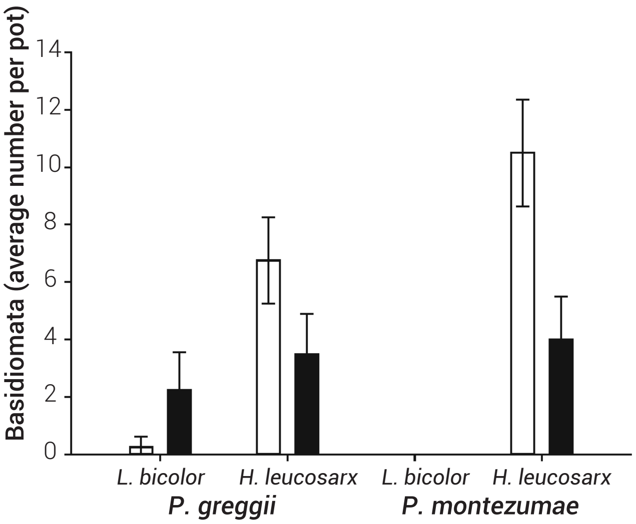

Figure 1. Basidiomata production during a 4-month period (October 2013 to January 2014) of two species of edible ectomycorrhizal fungi associated with two neo-tropical trees in their 6th year of growth, covered with a yellow (hollow columns) or a red (filled columns) filter. Vertical bars indicate 95 % confidence limits.

DISCUSSION

This study found an abundant formation of mature fertile basidiomata of *L. bicolor* and *H. leucosarx* associated with around 6-year-old neo-tropical *P. greggii* and *P. montezumae* seedlings, using ground pilei containing spores as inoculum source. There have been previous reports of basidiomata formation in several edible ectomycorrhizal fungal species using mycelium as inoculum source: basidiomata of *H. cylindrosporum* have formed with *P. pinaster* (Debaud and Gay, 1987), *L. laccata* with *P. patula*, *L. bicolor* with *P. strobus* (Lamhamedi *et al.*, 1994) and *Lactarius deliciosus* with *P. sylvestris* (Guerin-Laguette *et al.*, 2000). Massicotte *et al.* (2005) reported the early stages of the ontogeny of *L. bicolor* basidiomata associated with *P. sylvestris* in solid medium; however, they did not report whether these basidiomata were fertile or not, but the features described are similar with regard to the shape of the mycelial aggregates and primordia found in the developmental pattern of *L. bicolor* basidiomata in the present study.

Basidiomata formation in ectomycorrhizal fungi is a complex phenomenon because a set of specific physical, nutritional and biotic factors is required, as well as the obligate dependency towards the host plant. In general, these conditions have been poorly studied in the case of edible ectomycorrhizal mushrooms (Kües and Liu, 2000; Wessels, 1993; Yamada *et al.*, 2007).

There is a previous report of primordium formation for *L. laccata*, associated with *P. sylvestris* and *P. resinous* (Massicotte *et al.*, 2005). In the genus *Hebeloma*, various studies have reported the fruiting phenomenon: Debaud *et al.* (1981) reported mycorrhizal synthesis between an arctic-alpine evergreen shrub, *Dryas octopetala* L., and *H. alpinum* and *H. marginatulum* (J. Favre) Bruchet, which fruited after eight months in culture. Debaud and Gay (1987) reported primordium formation in *H. cylindrosporum* associated with *P. pinaster*, a year and a half after inoculation in sterile conditions using peat moss and vermiculite as substrate. However, this fungus did not complete its life cycle in the absence of the host plant in the same culture conditions and only produced primordia.

Ohta (1998) succeeded in producing basidiomata, with spore formation, of *H. radicosum* and *Hebeloma* sp. [later on described as *H. radicosoides* Sagara, Hongo et Y. Murak. by Sagara *et al.* (2000)] in culture medium, and reported the nutrients that promote mycelial growth and basidiomata production for these two species. Here, the production of primordial and fertile basidiomata of *H. leucosarx* on inoculated trees is reported for the first time. The basidiomata formation of *Laccaria* and *Hebeloma* in the present study has a great biotechnological potential for the use of these species as inoculants of neo-tropical trees of forest importance. Indeed, these fungi are pioneer species that produce beneficial effects in terms of growth and nutrient content (N, P, K, Ca and Mg) of their associated hosts (Carrasco-Hernández *et al.*, 2011; Martínez-Reyes *et al.*, 2012) and are widely marketed for consumption in the markets of central and southeastern México (Pérez-Moreno *et al.*, 2008).

Several studies have shown that environmental conditions are crucial to basidioma formation in ectomycorrhizal fungi, including temperature, carbon dioxide concentrations in the atmosphere, moisture, salinity (Kües and Liu, 2000; Wessels, 1993), fertilization regime (Debaud and Gay, 1987; Godbout and Fortin, 1990), relative humidity (Yamada *et al.*, 2007), the contribution of host carbohydrates (Lamhamedi *et al.*, 1994), and the abundance of ectomycorrhizal colonization of the fungal species of interest (Guerin-Laguette *et al.*, 2013; 2014; Pilz and Molina, 2001).

It has also been shown that electric impulses promote basidiomata formation in *L. laccata*, because they cause ruptures in the hyphae or enzyme activation through the application of high voltage, which acts as a stimulator for basidiomata formation (Ohga and Ida, 2001; Ohga *et al.*, 2004; Takaki *et al.*, 2009a). Takaki *et al.* (2009a; 2009b; 2010), reported a similar trend in the production of the edible saprophytic mushrooms *Pholiota nameko*, *Lentinula edodes*, known as shiitake, and *Lyophyllun decastes* using electrical impulses.

Figure 2. General aspects of the experiment (a) sale of the edible mushrooms *Laccaria* spp. and *Hebeloma* spp. in Ozumba market, state of México; (b) *Pinus montezumae* (Pm) growing in 7200 cm^3 pots with red filter (left) and yellow filter (right); (c) whole root system of *Pinus greggii* with abundant external ectomycorrhizal mycelium of *Hebeloma leucosarx* (Hl) in 140 cm^3 pot; (d) ectomycorrhiza of Pm with Hl; (e-k) Basidiomata development of Hl associated with Pm: (e) mycelial aggregate with small primordia; (f) hemispherical primordia (arrow) and an immature basidioma (double arrow); (g) an immature basidioma with hemispherical pileum and short stipe; (h) almost matured basidiomata with elongated stipe; (i) a matured basidioma showing brownish gills; (j) a senescent basidioma, and dark brown spore deposit on the soil surface (arrow); (k) verrucose spores of Hl. Bars sizes: (b) 10 cm; (c) 3 cm; (e) 1 mm; (f) 0.5 cm; (h) 1.2 cm; (i) 4 cm; (j) 3 cm; (k) 10 µm).

The present study found that different light wavelengths (590 nm *versus* 660 nm) differentially influenced the number of *L. bicolor* and *H. leucosarx* basidiomata produced. Previously, Lamhamedi *et al.* (1994) showed that the higher the photosynthetic flux densities the bigger the size of basidiomata of *L. bicolor* associated with *P. strobus* L. Additionally, they showed that greater net photosynthesis produced higher basidiomata biomass; and that the removal of basidiomata resulted in a quick decrease of net photosynthesis and stomatal conductance of the host trees. It is generally known in saprotrophic *Coprinopsis* and *Lentinus* that different light intensities produce excellent basidiomata production (Ellis *et al.*, 1999; Kitamoto *et al.*, 1972; Kües, 2000).

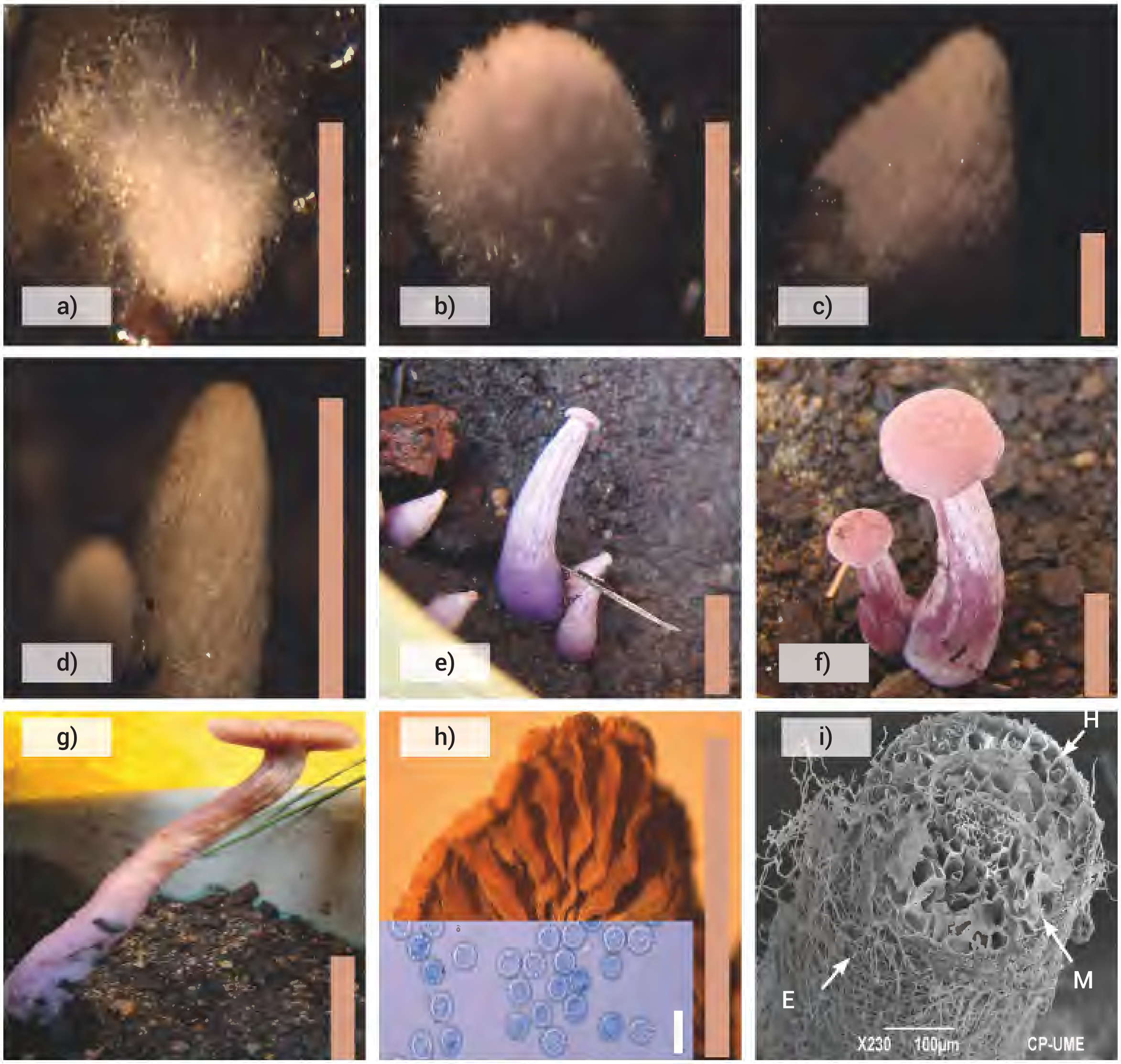

Figure 3. Developmental pattern of basidiomata of *Laccaria bicolor*. (a) Mycelial aggregate; (b) subspherical young primordium; (c) pyriform primordium; (d) subovoid primordium; (e) young basidiomata with initial pileus formation; (f) young basidiomata with subspherical pilei; (g) mature basidioma showing the characteristic violet color at the base of the stipe; (h) gills of a senescent basidioma (top) and globose spores showing their spiny ornamentation; (i) ectomycorrhizal roots of *P. greggii* with *L. bicolor*, showing external hyphae (EH), mantle (M) and Hartig net (Hn) with scanning electron microscopy. Bar sizes: (a) 500 µm; (b) 1 mm; (c) 2 mm; (d) 3 mm; (e) 1 cm; (f) 2 cm; (g) 1.75 cm; (h) 3 cm (for pileus) and 10 µm (for spores).

An interesting observation in the present study was the difference in the size of *L. bicolor* and *H. leucosarx* basidiomata in relation to the size of the containers. When the plants were in the 1000 cm^3 containers, the formation of *L. bicolor* basidiomata with a maximum height of 4 cm was observed, while the basidiomata formed in the 6-kg pots were up to 7 cm in height. Similarly, in the case of *H. leucosarx*, the basidiomata formed had a maximum height of 6 cm in the 1000 cm^3 containers, while in the 7200 cm^3 containers the basidiomata formed were up to 18 cm in height, which is their natural size when collected in the field.

One reason that could explain the smaller size of the basidiomata in the smaller containers could be a reduced supply of nutrients and water, as noted by Kikuchi *et al.* (2009). Another interesting observation was that the *L. bicolor* and *H. leucosarx* primordia obtained in our experiments sometimes developed at the base of the root system, near the drainage holes of the container. A similar phenomenon was reported for *C. cibarius* inoculated to *P.*

sylvestris Danell and Camacho (1997), and for *L. bicolor* primordia associated with white pine seedlings (Godbout and Fortin, 1990). In our case, basidiomata did not show positive gravitropism when they grew near the drainage holes as their stipes remained upright with their pilei at the bottom. Guerin-Laguette *et al.* (2000) also observed the lack of positive gravitropism with *L. deliciosus* growing upside down underneath containerized, mycorrhized, *P. sylvestris* seedlings.

CONCLUSIONS

To the best of our knowledge, this study demonstrates for the first time that light wavelength is a factor which influences basidiomata formation in ectomycorrhizal fungi, light wavelength differentially influencing the basidiomata formation depending on the fungal species. For *H. leucosarx*, a statistically higher number of basidiomata was observed when the pots were covered with a yellow filter, compared to a red filter, regardless of the associated tree species. An opposite trend was observed for *L. bicolor*, where a statistically higher number of basidiomata was recorded in *P. greggii* covered with a red filter.

Additionally, the host tree species influenced basidiomata formation differentially. The number of basidiomata of *H. leucosarx* associated with *P. montezumae* was higher than the number of basidiomata recorded in association with *P. greggii.* In the case of *L. bicolor*, basidiomata were only recorded when it was associated with *P. greggii*. Finally, the developmental process of *H. leucosarx* basidiomata is described for the first time. The developmental process of both *L. bicolor* and *H. leucosarx* basidiomata was recorded in detail.

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