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Mycorrhizal inoculation of pecan seedlings with some marketable truffles

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Pecan is the common name of *Carya illinoinensis* (Wangenh.) K. Koch, an ectomycorrhizal tree native to North America, also frequently known as hickory. Mycorrhizal inoculations of pecan seedlings with: *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. indicum* Cooke & Massee, and *T. lyonii* Butters are described and discussed.

Key words: *Carya illinoinensis*, truffle spore-slurry, multi-cropping, *Tuber borchii, T. aestivum, T. indicum, T. lyonii*

INTRODUCTION

Pecan is the common name of *Carya illinoinensis* (Wangenh.) K. Koch, also frequently known as hickory. It is a deciduous tree in the Juglandaceae family with pinnately compound leaves, which produces large and economically valuable nuts. This tree also establishes ectomycorrhizal (ECM) associations with hypogeous and epigeous fungi. Pecan is native to North America and grows naturally on the moist bottomland habitat along streams in the USA ranging from Indiana south to Kentucky and Alabama and from Iowa south to Texas, principally along the Mississippi river.

This species is cultivated largely in southeastern North America exclusively for the edible nuts, which are used for pecan pie, pecan pralines or food products including cereals, energy bars and candy bars. Pecan production of the United States it is likely to be over 433 million dollar per year, and the industry is in a period of growth (USDA 2008). A large proportion of pecan harvests are being exported to China, a rapidly growing market. Large productive commercial pecan orchards have also been recently established in Brazil, Israel, and Australia (Wakeling et al. 2001).

In the late 1980's Hanlin and colleagues (1989) discovered truffles fruiting naturally in pecan orchards in Georgia, USA. Bonito and colleagues (2011) followed up on this study and showed that the ECM truffle genus *Tuber* (*Pezizaceae*) is naturally abundant in fungal communities of many pecan orchards in Georgia, including the species *Tuber lyonii* Butters, whose ascocarps are frequently found and collected. Although *T. lyonii* is sold and served in US restaurant with success, the market is still underdeveloped because of its low organoleptic qualities, low availability and low quality of truffles collected (Bonito et al. 2011; Benucci et al. 2012a). Truffles are harvested by raking, then mature and less aromatic immature truffles are marketed spreading a negative opinion on the product.

MATERIAL AND METHODS

To overcome these problems and evaluate whether pecan can associate with both native and non-native truffle species as first step of future potential multi-cropping of pecans and truffles, some investigation on ECM inoculations with *Tuber* spp. on pecan have been realized (Bonito et al. 2010; Bonito et al. 2011; Benucci et al. 2012a; Bonito et al. 2012). Spore inocula were prepared with appreciated and widely distributed truffle species: *Tuber aestivum* Vittad., *Tuber borchii* Vittad., *Tuber indicum* Cooke & Massee, *Tuber macrosporum* Vittad. and *Tuber lyonii* Butters.

After inoculations with spore slurries, pecan seedlings were grown in greenhouse conditions for several months (Fig. 1a), after which plants were harvested and mycorrhization levels quantified visually (Bencivenga et al. 1995). ECM were described morpho-anatomically and molecularly by species-specific polymerase chain reactions (PCRs) (Mello et al. 1999; Mello et al. 2002) and/or by sequencing and analyzing the internal transcribed spacer (ITS) (White 1990) and 28S large subunit (LSU) (Vilgalys, Hester 1990) nuclear ribosomal DNA (nrDNA) region.

RESULTS

Tuber borchii and *T. aestivum* formed abundant ECM on pecan (Fig. 1c, 1e), with ≈60% and ≈40% root-tip colonization, respectively (Benucci et al. 2012a). In contrast, no ECM of *T. macrosporum* were detected on pecan, which may indicate that this host is not compatible with this fungal species, although it is known that *T. macrosporum* ECM are difficult to obtain under laboratory and nursery conditions (Benucci et al. 2012b). Morphological and anatomical characteristics of *T. borchii* and *T. aestivum* on pecan have similar features to those that they display with other host species (e.g., oak, hazelnut), and their morphologies are consistent with previous reports (Giomaro et al. 2000; Granetti et al. 2005). In particular, *T. borchii* possessed straight needle-shaped cystidia and puzzle-like cell pattern (Fig. 1g, 1h), while *T. aestivum* possessed curly cystidia and angular cell pattern (Fig. 1f, 1i).

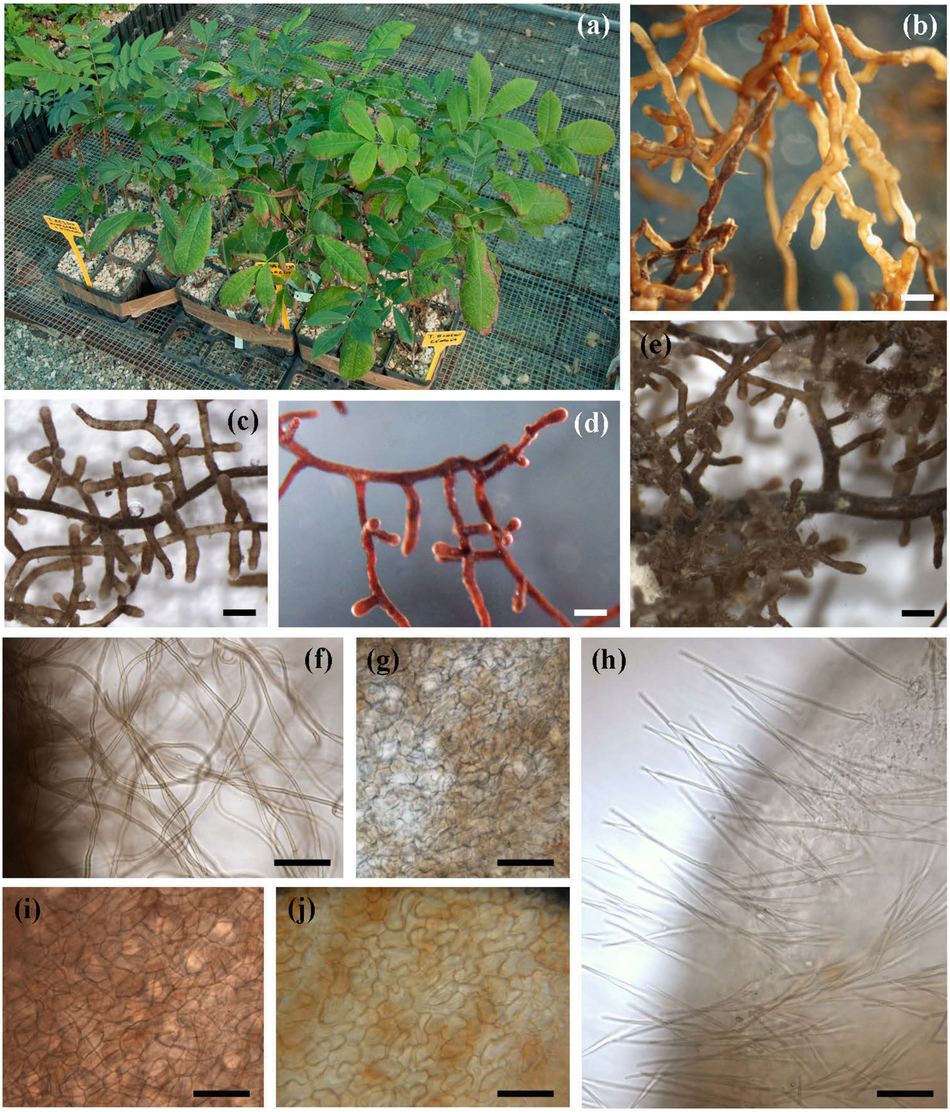

Fig. 1. Pecan seedlings and morphology of *T. aestivum*, *T. borchii*, *T. indicum* and *T. lyonii* ECM. (a) Pecan seedlings in the nursery; (b) *T. lyonii* ECM on pecan roots (scale bar=700 μm); (c) *T. borchii* ECM on pecan roots (scale bar=700 μm); (d) *T. indicum* ECM on pecan roots (scale bar=700 μm); (e) *T. aestivum* ECM on pecan roots (scale bar=700 μm); (f) curly cystidia of *T. aestivum* ECM (scale bar=30 μm); (g) outer mantle layer of *T. borchii* ECM (scale bar=30 μm); (h) needle-shape cystidia of *T. borchii* (scale bar=30 μm); (i) outer mantle layer of *T. aestivum* ECM (scale bar=30 μm); (j) outer mantle layer of *T. lyonii* ECM (scale bar=30 μm).

The molecular PCR assay confirmed that amplicons obtained from truffle ECM are consistent with our morphological and taxonomical assessment. Their ITS nrDNA sequences matched at 100% query coverage with 0.0 E-values and 99-100% identity to those present in GenBank for the same fungal species.

Bonito and colleagues (2010) were able to synthesize *T. indicum* ECM on *Carya illinoinensis* seedlings using standard inoculation methods. The identification of ECM was confirmed by morphology (Fig. 1d) and ITS sequence data. ECM produced by *T. indicum* on pecan were unramified to irregularly pinnate, dark amber in colour and showed characteristic puzzle-like pseudoparenchyma cells in the outer mantle with emanating right-angle branching cystidia, as reported from other hosts (Geng et al. 2009).

Tuber lyonii ECM were also well formed on pecan seedlings 6 months after they had been inoculated with *T. lyonii* spores (Bonito et al. 2011). Ectomycorrhizas are characterized by a thin and smooth mantle with epidermoid puzzle-like cells (Fig. 1b, 1j). Molecular ITS sequences confirmed the identity of *T. lyonii* ECM.

DISCUSSION

Together these data demonstrate that European, Asian and American truffles are able to form healthy ECM on pecan, regardless of the fact that this plant is endemic to North America. These studies lead us to conclude that there is the potential to multi-crop various truffle species with pecan. To successfully cultivate pecan with the simultaneous production of truffles represents a unique opportunity to diversify agricultural outputs from a given tract of land, and to add extra income in an environmental and sustainable way (Benucci et al. 2013; Donnini et al. 2013).

Furthermore, given the attributes of pecan wood for lumber and flooring, opportunities exist to obtain additional revenue streams from the timber production at the end of the cultivation cycle or following stand thinning (Benucci et al. 2012a). In US, although some orchards were originally brought for lumber and furniture, most pecan trees taken out are chipped and used for smoking meats in fires and traditional barbeques. However, the wood is quite dense, making it hard on saw blades of modern mill equipment, and transport costs are high. Nonetheless, once CO_2 is regulated in the US, we expect more sustainable practices will develop and pecan wood and timber will be favored again for flooring and furniture.

CONCLUSIONS

In conclusion, given the ability of pecan to readily for ECM with economic truffle species from five different clades *Tuber* that includes North American, European, and Asian truffle species, pecan holds promise as a host plant that is compatible with the co-cropping of truffles and nuts. Whether or not *Tuber* ECM are maintained when mycorrhized seedlings are planted out in an orchard setting, and whether these truffle species will fruit with pecan still need to be addressed however. Moreover, in humid climates pecan cultivation often requires heavy inputs of biocides to combat plant diseases and inputs of fertilizers to boost the production of nuts. Further studies are needed to address the potential accumulation of these harmful substances inside of truffles.

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