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A Late Pennsylvanian fungal leaf endophyte from Grand-Croix, France

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ABSTRACT

The pre-Cretaceous fossil record of leaf-inhabiting fungi is meagre. A structurally preserved pinnule fragment from the Upper Pennsylvanian of Grand-Croix (Saint-Étienne Basin, France) contains a fungus of uncertain affinity that occurs as an intracellular endophyte in the asymptomatic hypodermis. The fungus consists of branched, septate hyphae producing long-necked hyphal swellings and spherical to ovoid structures, probably conidia, which may occur singly or in short chains. This discovery provides new insights into the biology of leaf-inhabiting fungi some 300 Ma ago, and indicates that the mycological definition of fungal endophyte is difficult to apply to fossils. We suggest that, with fossils, the designation fungal endophyte should be used strictly in a descriptive sense for all fungi occurring within intact plant cells or tissues in which there are no visible disease symptoms.

1. Introduction

Fungal endophytes are defined functionally by their occurrence within asymptomatic (i.e. lacking visible disease symptoms at the moment of detection; see Schulz and Boyle, 2005) tissues of plants (but see Hyde and Soytong, 2008, for alternative definitions). They occur in all major lineages of land plants and in communities ranging from the arctic to the tropics (Arnold et al., 2000; Arnold, 2007). Fungal endophytes may enter into varied and variable interactions with their hosts, including mutualism, cryptic commensalism, and latent and virulent pathogenicity (Schulz and Boyle, 2005, and references therein), in which they may significantly affect various degrees of host performance (e.g., Omacini et al., 2001; Arnold et al., 2003; Omacini et al., 2006; Arnold and Engelbrecht, 2007; Rodriguez et al., 2009). With this recognition of the ecological importance of fungal endophytes today, a more complete understanding of the occurrence and diversity of these organisms in the fossil record is pivotal to assessing how fungi may have functioned in shaping ecosystems of the past.

Leaf endophytes represent a significant proportion of today's total fungal diversity (Petrini, 1991; Arnold et al., 2000, 2001; Wang et al., 2009). Since all major lineages of fungi, as well as the endophytic life style in fungi, were in existence in the Early Devonian (e.g., Heckman et al., 2001; Taylor and Krings, 2005), well before leaves evolved, it appears probable that leaf-inhabiting endophytic fungi occurred shortly after leaves had evolved. It is interesting, however, that fossil evidence for fungi in leaves almost exclusively comes from the Cretaceous and Cenozoic (e.g., Meschinelli, 1898; Le Page et al., 1994; Van der Ham and Dortangs, 2005; Jasinski and Payette, 2007). Pre-Cretaceous records are exceedingly rare (e.g., Oliver, 1903; Barthel, 1961; Krings, 2001) despite the fact that late Paleozoic and Mesozoic foliage fossils have been studied for a long time. This may be due in part to the inability to recognize the fungi (e.g., in impression and compression fossils), or lack of interest in such microorganisms. However, certain secondary metabolites produced by the plants, as well as physical constraints imposed by the environment, may have also played a role in defining the extent of fungal colonization in ancient ecosystems (Taylor and Osborn, 1996).

The Upper Pennsylvanian cherts from Grand-Croix in central France contain numerous well preserved vascular plants (Galtier, 2008). Associated with the plants is a diverse assemblage of fungi. This paper offers evidence of a fungal leaf endophyte from Grand-Croix that occurs in the tissues of what we believe is a pecopterid fern pinnule fragment. This discovery adds new information about the morphology and biology of endophytic fungi in a paleoecosystem ~300 Ma ago.

2. Material and methods

The Grand-Croix cherts come from the eastern part of the Saint-Étienne Basin in central France where they occur within the "Poudingue mosaïque" at several localities in the vicinity of the city

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of La Grand-Croix (for details, see Doubinger et al., 1995; Galtier, 2008). The exact stratigraphic provenance and age of the cherts remain controversial. They have been interpreted as belonging either to the terminal portion of the Rive de Gier Formation, which has been dated as Stephanian A (Vetter, 1971), or to the overlying deposits, which are earliest Stephanian B in age (Pruvost, 1947). Nevertheless, the plants contained in the Grand-Croix cherts are probably reworked, and hence slightly older, i.e. late Stephanian A (equivalent to the late Barruelian) in age (see Galtier and Scott, 1981; Doubinger et al., 1995). The flora from Grand-Croix represents one of only a few European equivalents to the Late Pennsylvanian coal ball floras from North America (Galtier and Phillips, 1985; Galtier, 2008).

The infected pinnule fragment (in transverse section) was identified in a thin section prepared by cementing a piece of chert to a glass slide and then grinding the wafer until it is thin enough to be examined in transmitted light. The thin section was prepared by Bernard Renault during the late 19th century, and is today housed in the Muséum National d'Histoire Naturelle (Laboratoire de Paléontologie, 'collection Renault') in Paris (France) under accession number REN-2/40 ('collection Renault' slide 40 in box 2).

3. Description

The fungus occurs in approximately 40% of the preserved hypodermal cells of what we believe is a pecopterid pinnule. The organism has not been detected in other tissues of the pinnule, nor has it been found in other plant fossils from Grand-Croix or in the chert matrix.

The section through the pinnule fragment (Plate I, 1) shows the upper epidermis composed of relatively small cells up to 35 µm high, a partially preserved two- to three-layered hypodermis of variously sized and shaped (but mostly ovoid or isodiametric and up to 90 µm in diameter) cells, and a multi-layered palisade parenchyma composed of rod-shaped cells up to 20 µm wide and 150 µm long. A subepidermal secretory cavity with contents occurs as an opaque ovoid body [SC] between the hypodermis and palisade parenchyma.

The endophyte (Plate I, 2–8) consists of septate hyphae, (2–)2.5–4 µm wide, that are thin-walled (Plate I, 3–5); irregular hyphal constrictions may occasionally occur (Plate I, 3, 7). Hyphae may be unbranched or branched. Branches may be distinctly narrower (Plate I, 3 [black arrow], 5 [black arrow]) or with approximately the same width as the hypha from which they extend (Plate I, 3). Narrower hyphal branches are up to 1.5 µm wide. Hyphae usually extend vertically (e.g., Plate I, 5), but some may also assume a more horizontal course, usually extending along the host cell walls (Plate I, 7). Hyphae penetrating host cell walls form an indistinct bulb-like swelling (up to 4.5 µm wide) close to where they enter the wall (Plate I, 4 [white arrow]). A single hypha that terminates in (or extends from) a small, irregular and thick-walled structure, which is separated from the hypha by a septum, is illustrated in Plate I, 4 [black arrow].

Prominent hyphal swellings with long conical necks, up to 25(–40) µm long (including neck) and 7–10(–15) µm wide, sometimes occur terminally on short hyphal branches (Plate I, 5 [in upper host cell], 8). They are separated from the parental hypha by a simple septum at the base of the neck (Plate I, 8 [black arrow]). The conical necks may be straight (Plate I, 8) or variously curved (Plate 1, 5 [in upper host cell]). Also produced by the fungus are spherical to ovoid structures, up to (7–)9 µm long and (5.5–)7 µm wide (Plate I, 5–8). These may occur singly or in short chains of two to four, and may arise laterally from normal hyphae (Plate I, 7 [white arrow]), from the tips of short hyphal branches (Plate I, 7 [black arrow]), or laterally from the long-necked hyphal swellings (Plate I, 8). Spherical-ovoid structures of this type have not been found isolated in any of the host cells. A simple septum is present between the spherical-ovoid structures and the parental hypha or hyphal swelling, as well as between the individual spherical-ovoid structures of a chain; in a few instances, however, the septum appears to be nearly inconspicuous or lacking (e.g., Plate I, 8 [white arrow]). Some host cells contain clusters of hyphal swellings and/or spherical-ovoid structures. These clusters typically occur in the upper (i.e. pointing towards the epidermis) portion of the host cell (Plate I, 5, 6); only one relatively small hypodermal cell appears to be occupied entirely by the fungus (Plate I, 2).

Observable host reactions caused by the fungus are not present, with one possible exception. The hypodermal cell illustrated in Plate I, 4 contains several hyphae that penetrate the cell wall. It is interesting to note that the epidermal cell above is filled with opaque matter (see Plate I, 1 [arrow]), which could either represent a host response or preservational artefact.

4. Discussion

The fungus described here represents only the second record of fungi in land plant leaves from Grand-Croix. The other leaf-inhabiting fungus from this locality occurs in the form of small pockets containing spores on the abaxial side of a pinnule of *Alethopteris aquilina* (Schlotheim) Göppert. The pockets were initially believed to represent sporangia (Renault, 1883), but later reinterpreted as fungal reproductive structures (Oliver, 1903), and eventually named *Urophlyctites oliveranus* (Magnus, 1903). Unfortunately, we have not yet been able to locate the original specimen in the 'collection Renault' in Paris, and thus the nature of this fossil remains inconclusive. Although the evidence for leaf-inhabiting fungi from Grand-Croix is not widely established, the few occurrences do indicate that these fungi were present, and hence may have been important in the functioning of this paleoecosystem ~300 Ma ago.

4.1. Fungal endophytes: the trouble with fossils

The term fungal endophyte is generally used to refer to all fungi that thrive within living plant tissues without causing visible disease symptoms at the moment of detection. In this context, colonization may be inter- or intracellular, localized or systemic (Schulz and Boyle, 2005). Accordingly, the fungus from Grand-Croix satisfies the definition of an endophyte because the host does not demonstrate disease symptoms (e.g., local necroses, hypertrophy, abnormal tissue growth). Moreover,

Plate I. Fungal leaf endophyte from the Upper Pennsylvanian of Grand-Croix, France; all specimens from slide REN-2/40 ('collection Renault', Paris).

Fig. 1: Pinnule fragment showing the upper epidermis, hypodermis, palisade parenchyma, and a secretion body [SC]; arrow indicates epidermal cell filled with opaque matter; bar = 100 µm.
Fig. 2: Detail of Plate 1, 1 (different focal plane), focusing on some of the epidermal [E] and hypodermal cells, the latter containing the fungus; bar = 15 µm.
Fig. 3: Branched hypha; white arrows indicate septa, black arrow indicates narrow hyphal branch; bar = 10 µm.
Fig. 4: Detail of Plate 1, 1, focusing on hyphae in a hypodermal cell immediately below the epidermis; white arrow indicates indistinct bulb-like swelling of hypha penetrating the host cell wall, black arrow shows irregular hyphal thickening; bar = 10 µm.
Fig. 5: Hypha extending through two hypodermal cells; note that the hypha produces spherical/ovoid structures and hyphal swellings only in the upper portion (closest to pinnule surface) of the cells, immediately before it enters the next cell; arrow indicates narrow hyphal branch; bar = 15 µm.
Fig. 6: Detail of Plate 1, 5 (different focal plane), focusing on the lower cluster of spherical/ovoid structures; note that the structures occur in short chains; bar = 15 µm.
Fig. 7: Hypodermal cell containing hyphae and spherical-ovoid structures; white arrow indicates ovoid structure positioned laterally on hypha, black arrow indicates ovoid structure positioned terminally on short hyphal branch; bar = 15 µm.
Fig. 8: Hyphae, hyphal swellings, and spherical-ovoid structures in a host cell; white arrow indicates connection between hyphal swelling and ovoid structure (note absence of septum), black arrow indicates septum at the base of hyphal swelling; bar = 10 µm.