SHORT COMMUNICATION

Biscogniauxia nummularia infecting beech (Fagus sylvatica) trees and sympatric plants of the sedge Carex brevicollis

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Summary

Biscogniauxia nummularia is known for its association with beech (Fagus sylvatica), on which it occurs as a saprotroph and a pathogen causing strip cankers following water stress. This fungus has also been reported as a dominant endophytic species in plants of the sedge Carex brevicollis growing in the understory of beech forests and adjacent grasslands in Sierra de Urbasa (Navarre, Spain). In this area, stromata of B. nummularia were observed in dead and living wood of beech trees at several locations where plants of C. brevicollis also contained the fungus as an endophyte. Pure cultures obtained from stromata of B. nummularia on F. sylvatica trees were compared to endophytic isolates from symptomless C. brevicollis. Culture morphology and micromorphology as well as rDNA sequences of the internal transcribed spacer (ITS) regions were identical, suggesting that B. nummularia from beech can also live endophytically in C. brevicollis. It is unknown whether the endophytic strains of Carex might have a role as an inoculum source for the infection of beech trees, or whether they represent a dead end in the life cycle of the fungus.

1 Introduction

Biscogniauxia nummularia (Bull.: Fr) Kuntze is a member of the Xylariaceae, a family that includes saprobes, pathogens and endophytes (Petrini and Petrini 1985; Ju et al. 1998). This fungus occurs as an endophyte in several species belonging to different plant families, but seems to produce sexual reproductive structures exclusively on beech (Fagus sp.) (Petrini and Petrini 1985; Nugent et al. 2005). These fructifications are dark stromata that develop on dead and living beech branches and trunks (Fig. 1a) (Hendry et al. 1998; Ju et al. 1998). Although B. nummularia is mainly saprotrophic, it can behave as a pathogen under some circumstances, being able to form elongated cankers that can kill branches, trunks and trees. This disease, called strip cankerling, is associated with declines observed in European beech (Fagus sylvatica) woods, following episodes of drought stress (Hendry et al. 1998; Granata and Sidoti 2004; Nugent et al. 2005).

In a survey of fungal endophytes occurring in leaves of the sedge Carex brevicollis (DC.), (Fam. Cyperaceae) in Sierra de Urbasa (Navarre, Spain), plants were sampled at two different locations, Udau and Bardoitza. These two sites, separated 5.5 km in linear distance, were chosen because in each of them, C. brevicollis occurs in two adjacent habitats, in grasslands and in beech forest understory. At each location, 10 plants were sampled from a grassland, and another 10 from the beech forest understory. In that survey, B. nummularia was found to be the dominant endophytic species at both locations and habitats, being detected in 80% of these 40 plants (Canals et al. 2014).

We were interested in knowing whether this fungus was present in beech trees at the locations where infected C. brevicollis plants had been reported, and to compare the fungal strains obtained from both host plants.

2 Materials and methods

Sierra de Urbasa, the study site, is a mountainous rangeland located south of the western Pyrenees (950 m a.s.l.). This area is characterized by a karstic landscape covered by 11 400 ha of grasslands, heathlands and beech forests where the sedge develops in both open and closed habitats. According to flora catalogues, C. brevicollis is a common component of stony and calcareous grasslands in mountainous areas of West Asia and Central and South Europe (Chater 1980), and in Sierra de Urbasa, it is a common species in the understory of xerophilous beech forests.

We surveyed beech trees for the presence of B. nummularia stromata at several locations in Sierra de Urbasa, including those two (Udau and Bardoitza) where B. nummularia was isolated from asymptomatic C. brevicollis plants occurring in grasslands and in the adjacent beech forest understory (Canals et al. 2014). At the above-mentioned and some other locations (i.e. Ubaba), we observed dark stromata embedded in the bark of dead wood, but also in living branches (Fig. 1a). Six more plants of C. brevicollis were sampled at an additional location (Tximista) in Sierra de Urbasa and processed for endophyte isolation as described by Canals et al. (2014).

To determine whether the fungal strains infecting C. brevicollis and F. sylvatica belonged to the same species, morphological and molecular characters of cultures obtained from both host plants were compared. To obtain pure cultures from stromata present in beech branches, small pieces of stroma were placed inside a Petri plate on top of humid filter paper, and after two days, dark ovoid ascospores were ejected to the cover of the plate (Fig. 1b). Some ascospores were streaked on the surface of a water agar plate, and individual germinated spores were picked to obtain pure cultures. Cultures
obtained from both host plants were identified using macroscopic and microscopic (conidiophore) characters (Petrini and Petrini 1985), as well as the nucleotide sequence of their ITS1-5.8S rRNA-ITS2 region. The methods described by Sánchez Marquez et al. (2007) were used to amplify and sequence the fungal DNA. Genetic distances between strains were estimated from alignments, and a neighbour-joining tree was drawn including sequences of reference strains of *B. nummularia* and *B. mediterranea* using Mega Software (Tamura et al. 2013).

### 3 Results and discussion

*Biscogniauxia nummularia* was isolated from five of the six *C. brevicollis* plants sampled at Tximista. The ease of finding *B. nummularia* in *C. brevicollis*, together with the results of the previous survey, where the fungus was found in 80% of the plants (Canals et al. 2014), suggests that there is a high prevalence of *B. nummularia* as an endophyte in leaves of *C. brevicollis* in Sierra de Urbasa. The cultures obtained from *C. brevicollis* leaves or from beech stromata were similar in potato dextrose agar, having a dark tan colour in their surface and aerial mycelium. Microscopic observation of PDA cultures allowed to observe *Periconiella*-like conidiophores, the anamorphic state of *Biscogniauxia* (Petrini and Petrini 1985) (Fig. 1c–d). Therefore, both the culture macroscopic characteristics and the conidiophore morphology suggested that the fungus isolated from both hosts was *B. nummularia*.

The phenotypic identification of cultures was confirmed by the analysis of nucleotide sequences from three strains obtained from *C. brevicollis* leaves, and two strains obtained from beech stromata. These five sequences (EMBL nucleotide database accession numbers LN829124–LN824128) were identical when aligned. A neighbour-joining tree made with the distances estimated from the alignment of these and reference sequences of *B. nummularia* and related taxa placed both types of cultures within a *B. nummularia* clade (Fig. 2). Identity of morphology and ITS sequences is strong evidence that the *B. nummularia* isolates from beech and *C. brevicollis* belong to one and the same fungal species. However, cross-inoculation experiments would be needed for final proof that *B. nummularia* isolates from beech can infect *C. brevicollis* and vice versa.

The endophytic infection of *C. brevicollis* by *B. nummularia* plants did not seem to be permanent or systemic, because the fungus could not be isolated from new leaves produced by any of six previously infected plants that had been maintained in a glasshouse in Salamanca, far away from their natural habitat, and from beech trees.

Although it is claimed that *Biscogniauxia* taxa are exclusive parasites of dicotyledous angiosperms (Ju et al. 1998), Petrini and Petrini (1985) have detected *B. nummularia* as an endophyte in some species of grasses. The results of the
The present study shows that *B. nummularia* can infect two host plant species that live in sympatry, a small monocotyledonous plant (*C. brevicollis*) and a tree (*F. sylvatica*). As an endophyte, this fungus is a host generalist, but its capability to develop a teleomorphic state and sexual ascospores appears to be narrow and possibly limited to beech and a few other tree species (Petrini and Petrini 1985). This characteristic of being generalist endophytes with a narrower host range for fructification is common to several other xylariaceous taxa (Petrini and Petrini 1985; Sieber 2007; Osono et al. 2013). Therefore, it is likely that the ascospores ejected from stromata in beech trees do infect *C. brevicollis* plants. But it is unknown whether the fungus can produce conidia or other inoculum in *C. brevicollis* plants. Some endophytes sporulate on senescing and/or dead host tissue (Vázquez de Aldana et al. 2013). If this were also the case for endophytic *B. nummularia* in *C. brevicollis*, the endophytic phase in *C. brevicollis* could play a role as a reservoir for additional inoculum of the strip canker disease caused by this fungus on beech. Alternatively, the fungus might be unable to sporulate in its herbaceous host, and in this case, the endophytic infection of *C. brevicollis* would be a dead end in the life cycle of the fungus.

**Acknowledgements**

We thank the authorities of the Natural Park Urbasa-Andia, for allowing us to do the field sampling of this work.

**References**


