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Response of endophytic *Biscogniauxia mediterranea* to variation in leaf water potential of *Quercus cerris*

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Summary

Endophytic behaviour of *Biscogniauxia mediterranea*, the causal agent of charcoal disease of oak, was studied over two growing seasons on *Quercus cerris* in a forest in central Italy. Isolation of the fungus from asymptomatic tissues varied with tissue type, period of sampling and year. Presence of the fungus in asymptomatic plants was significantly higher in fall, compared with that in spring and summer. However, a significant effect of tissue water content on the presence of *B. mediterranea* was recognized. The results suggest that proliferation of *B. mediterranea* is favoured during the endophytic phase by a decrease in host water potential.

1 Introduction

The effects of climate change on forest ecosystems on the local and/or global scales are major issues for the scientific community. Increasing mean temperatures and variance are likely to result in an intensification of extreme events (Easterling et al. 2000), i.e. flooding or drought, that could bring unpredictable changes in species assemblages, among-species competition and potential for survival over time of a number of forest ecotypes. Plant–fungal communities actively participate in these changes through modifications in ecological behaviour, i.e. saprotrophic and symbiotic, with evident consequences for the plant hosts. Many microfungi could be considered sensitive bio-indicators of climatic change because of their ability to respond rapidly to changes in temperature, humidity or host physiology, in terms of population size, reproduction and dispersal. Some secondary plant pathogenic fungi with endophytic behaviour are particularly sensitive to variations in host physiology driven by stress conditions (Paoletti et al. 2001; Desprez-Loustau et al. 2006; Capretti and Battisti 2007). Among these *Biscogniauxia mediterranea*, the causal agent of the charcoal disease of oak, exists for part of its life cycle as an endophyte in host tissues including twigs, bark, leaves and, to a lesser extent, wood (Collado et al. 2001; Mazzaglia et al. 2001). In hosts subjected to environmental stress *B. mediterranea* is able to rapidly colonize the xylem and bark tissues, induce necrosis and canker formation, and accelerate tree decline and eventually death (Desprez-Loustau et al. 2006; Capretti and Battisti 2007).

The necrotrophic activity of *B. mediterranea* and canker formation on water-stressed hosts was described previously (Vannini and Scarascia Mugnozza 1991; Vannini and Valentini 1994; Vannini et al. 1996). Its endophytic behaviour has been successively observed and studied (Collado et al. 2001; Mazzaglia et al. 2001; Luchi et al. 2005), with particular regard to its cryptic presence in different tissues and seasonal frequency of isolation. These studies did not consider the effects of tissue water content on the endophytic behaviour of *B. mediterranea* in trees, in natural or naturalized forests.

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Outbreaks of charcoal disease and mass colonization by *B. mediterranea* occur on *Quercus cerris* when trees are subjected to conditions of severe water stress (Vannini and Scarascia Mugnozza 1991; Vannini et al. 1996). Whether *B. mediterranea* also responds to host tissue water potential gradients during the endophytic phase remains unknown. The aim of this work was to study the presence of endophytic *B. mediterranea* in tissues of *Q. cerris* at different water contents.

2 Materials and methods

The study site was located in a 35-year-old forest of *Q. cerris* in the Nature Reserve of Mt Rufeno in central Italy (42°49′N; 11°54′E) at an elevation of approx. 690 m a.s.l. This forest was previously reported to be affected by charcoal disease (Vannini and Scarascia Mugnozza 1991; Vannini et al. 1996). An experimental area of 2000 m^2 was delimited and all trees in the area were tagged. Fifteen trees, without evident signs and/or symptoms of charcoal disease, were randomly selected in the area.

Predawn leaf water potential (PWP) and midday leaf water potential (MWP) of trees were measured twice a month from June to September on three shoots for each of the 15 sample trees, using a Scholander–Hammel pressure chamber (PMS Instruments, Albany, OR, USA). Sampling of plant material was carried out in May, August and November 2002 and 2003. Samples of current year shoots (n = 8), bark (n = 16) and all xylem (n = 16) of 2-to 3-year-old branches, and leaves (n = 32) were collected from each of the 15 trees. Four fragments for each leaf were sampled at the four cardinal points. Samples were surface sterilized using the method of Fisher et al. (1986) for isolation of fungal endophytes from plant tissues, by immersing in 96% ethanol (1 min), sodium hypochlorite (6% available chlorine, 3 min) and 96% ethanol (0.5 min). Immediately after surface sterilization, samples were rinsed five times in sterile, distilled water and plated on potato dextrose agar (PDA; Difco, Detroit, MI, USA) adding streptomycin (0.06 g/l). Petri dishes were incubated at 30°C in the dark (Vannini and Scarascia Mugnozza 1991). A total of 1080 tissue samples per period were processed. Isolation frequency of *B. mediterranea* was calculated on the basis of the total number of isolations per tissue and season.

Colonies of *B. mediterranea* were identified morphologically using the reference strain ATCC 90363. Molecular identification was carried out according to Mazzaglia et al. (2001) using the primers MED1 and MED2 specific for *B. mediterranea*.

Linear regression analysis, t-tests and two-way ANOVA were carried out with the Prism4 package (GraphPad Software Inc., San Diego, CA, USA). Data sets were previously tested for normality with the Kolmogorov–Smirnov test included in Prism4.

3 Results

The values of PWP and MWP in 2002 and 2003 are shown in Fig. 1. MWP values in August, September and October 2003 were significantly lower than those in 2002 in the same months (unpaired t-test, $p < 0.0001$, <0.02 and <0.0001 respectively). PWP values in August and October 2003 were significantly lower than that in 2002 (unpaired t-test, $p < 0.0001$). Isolation of *B. mediterranea* in May, August and November 2002 and 2003 is shown in Fig. 2. 'Period of sampling' accounted for 53.8% of the total variance (two-way ANOVA, $F = 60.5$, $p < 0.0001$), whereas 'year' accounted for 6.65% of the total variance ($F = 14.8$, $p < 0.0002$). In both 2002 and 2003, sampling in November yielded a significantly higher percentage of isolations than sampling in May (Bonferroni post-test, $p < 0.001$) and August ($p < 0.001$). The Period of sampling had no effect between years ($F = 0.1$, $p > 0.05$, no interaction).

Figure 3 shows the isolation frequency of *B. mediterranea* from different tissues as a proportion of the total isolation frequency in 2003. The period of sampling accounted for

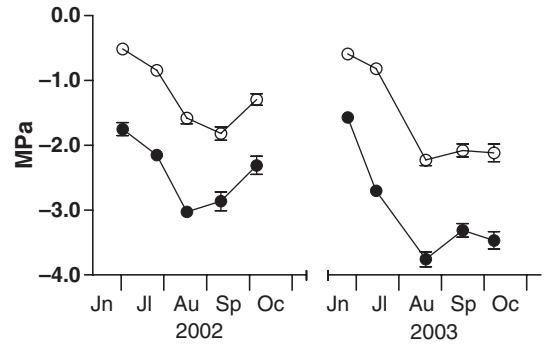

Fig. 1. Values of predawn (PWP) (○) and midday (MWP) (●) leaf water potential of *Quercus cerris* trees in 2002 and 2003. Vertical bars represent the standard error

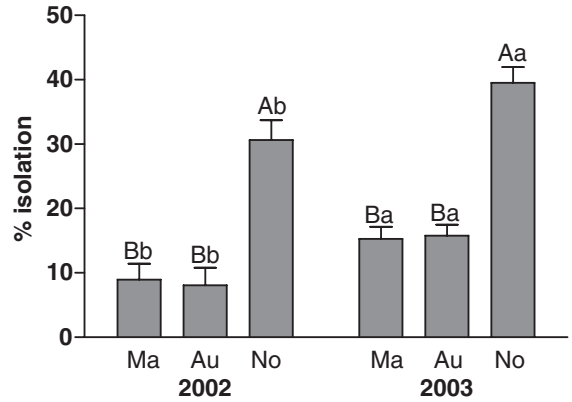

Fig. 2. Frequency of isolation of endophytic *Biscogniauxia mediterranea* from *Quercus cerris* tissues in May (Ma), August (Au) and November (No) 2002 and 2003. Same uppercase letter indicates no significant differences (Bonferroni post-test) between different months and within the same year. Same lowercase letter indicates no significant differences (Bonferroni post-test) between year and within the same month

<0.1% of the total variance ($F = 0.0$, $p > 0.05$; not significant). In contrast, the type of tissues accounted for 51.07% of total variance ($F = 109$, $p < 0.0001$); however, sampling season had a significant effect for each tissue ($F = 4.2$, $p < 0.0005$).

In Fig. 4a and b the seasonal MWP and PWP values are plotted against the frequency of isolation of *B. mediterranea* from each tree in November 2002 and 2003 respectively. A linear relationship was found between both MWP ($r^2 = 0.30$, $p < 0.05$) and PWP ($r^2 = 0.32$, $p < 0.05$) values and frequency of isolation of *B. mediterranea* in 2003. This relationship was not significant in 2002.

4 Discussion

This study re-emphasized the cryptic presence of endophytic *B. mediterranea* in different tissues and organs of *Q. cerris* and, uniquely, demonstrated for the first time that

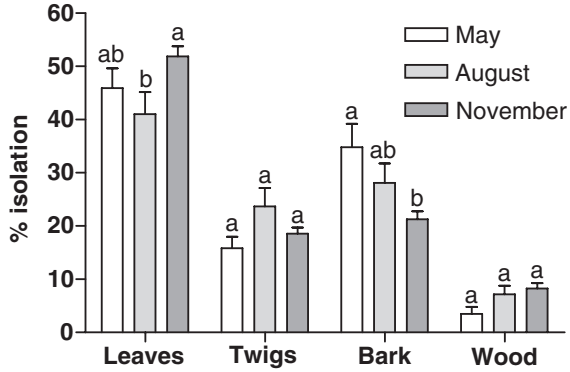

Fig. 3. Contribution of different host tissues to total isolation frequency of *Biscogniauxia mediterranea* in May, August and November. Same lowercase letter indicates no significant differences (Bonferroni post-test) between months and within the same tissue

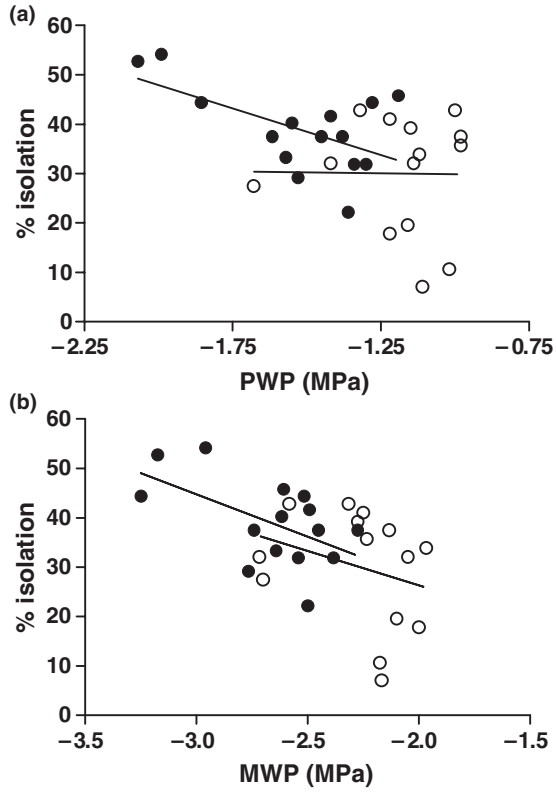

Fig. 4. Linear regression of seasonal (May to October) predawn (PWP) (a) and MWP (b), leaf water potential values *vs* isolation frequency of *Biscogniauxia mediterranea* from all tissue types in November for each of the *Quercus* cerris trees studied in 2002 (○) and 2003 (●)

proliferation of the endophyte is closely related to host phenology and to changes in the water content of tissues. As previously suggested (Hendry et al. 2002), the influence of wood water content on wood colonization by endophytic fungi in beech may be attributable to its effect upon the length of path for diffusion of respiratory gases, with increasing water content reducing the flux of oxygen to, and carbon dioxide from, respiring mycelia.

Biscogniauxia mediterranea accumulates in tissues of *Q. cerris* at the end of the growing season, as previously shown in *Quercus ilex* ssp. *ballota* (Collado et al. 2001. This behaviour could be related to the complex of biochemical and physiological changes occurring in plant tissues (Kozlowski et al. 1991) and organs (Verhoeff 1974) at the end of the growing season. Water content of tissues represents one of several interacting factors that could trigger the activity of *B. mediterranea*. Our results showed that during dry growing seasons, endophytic *B. mediterranea* has a better chance to proliferate in asymptomatic tissues of *Q. cerris*, supporting previous studies demonstrating the pathogenic ability of this fungus in causing canker and wood decay on severely water-stressed hosts (Vannini and Scarascia Mugnozza 1991).

In contrast to the report of Collado et al. (2001), *B. mediterranea* was detected in wood tissues of 2-to 3-year-old branches of *Q. cerris*. Contamination from bark may have occurred, although experimental conditions and sterilization procedures for samples used in this study were very strict, thereby limiting this risk. Although differences were not significant according to the Bonferroni post-test, an increase in the presence of *B. mediterranea* in wood, that paralleled a significant decrease in bark tissues, was observed from May to November 2003. This finding supports the hypothesis that the shift of *B. mediterranea* from the latent to the pathogenic phase includes the invasion of xylem tissues (Vannini and Valentini 1994). Chapela and Boddy (1988) provided direct experimental evidence for the influence of decreased water content in the initial development of fungi, including the xylariaceous *Hypoxylon fragiforme*, in the xylem of excised beech branches.

Release from quiescence of a latent fungal pathogen in response to physiological changes in the host (Stanosz et al. 2001) and the association with specific disease symptoms are difficult to demonstrate. This problem results from the fact that these organisms are constantly present in plant tissues (Faeth and Fagan 2002) and that more than one biotic and/or abiotic factor may be involved in triggering pathogenicity (Collado et al. 2001). Among the few studies addressing this problem, Stanosz et al. (2001) produced experimental evidence suggesting that the shift from the latent to the pathogenic phase in *Sphaeropsis sapinea* is driven by a decrease in the water potential of host tissues in *Pinus resinosa*. Further evidence was provided by Bassett and Fenn (1984), who noted proliferation of the stem canker pathogen *Hypoxylon atropunctatum* on girdled oak trees independent of inoculation, suggesting the presence of a latent inoculum of the fungus in host tissues. Direct evidence for the involvement of tissue water content in the passage of *B. mediterranea* from the latent to the pathogenic phase is lacking. However, the response of the endophytic population to tissue water potential strongly suggests that water shortage may play a role in this event. In addition, artificial inoculation of the fungus in bark tissues of plant submitted to different water regimes produced mass colonization of bark and woody tissues and symptoms of charcoal disease only on hosts with PWP values below −3.5 MPa (Vannini and Scarascia Mugnozza 1991; Vannini and Valentini 1994). PWP values below −3.5 MPa correspond to water stress conditions associated with severe loss in xylem conductivity because of embolism in *Q. cerris* (Vannini and Valentini 1994; D'Orazio 2000). These values were not reached in our study, where PWP never dropped below −2.2 MPa (August 2003). Thus the conditions in 2003 may not be sufficiently severe to cause mass colonization of hosts by *B. mediterranea* and appearance of symptoms and signs of charcoal disease.

As demonstrated in this study, average PWP and MWP values, and the related presence of endophytic *B. mediterranea*, differed among trees growing in the study area. Charcoal disease commonly appears as patches in the forest on single trees or groups of trees. Age, hierarchy and geographical position could account for increased colonization by *B. mediterranea* and eventually to the appearance of charcoal disease symptoms and signs. A geographical influence on distribution and abundance of fungal endophytes in tissues of woody species was also reported by Göre and Bucak (2007)for *Laurus nobilis* and by Collado et al. (1999) for *Q. ilex*.

Interactions between host plants and fungal endophytes may range from mutualism to antagonism depending on the endophyte and host genotype and environmental conditions (Faeth and Fagan 2002). In a scenario of global climatic change characterized by increases in temperature and extreme events, interactions between plants and fungal endophytes may undergo radical changes eventually in the direction of antagonism. An example of this phenomenon is the observation of the impact of *B. mediterranea* on oak forests in central and southern Italy following a decade of severe drought events; a change in the assemblage of oak species occurred, including an increase in the abundance of xerophytic species, such as *Quercus pubescens*, that were little impacted by the fungus (Vannini et al. 1996).

Acknowledgements

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