European Journal of Soil Biology 58 (2013) 42–44

Contents lists available at SciVerse ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

No allelopathic effect of the invader *Acacia dealbata* on the potential infectivity of arbuscular mycorrhizal fungi from native soils

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A R T I C L E I N F O

Article history:
Received 14 January 2013
Received in revised form
22 March 2013
Accepted 2 June 2013
Available online 21 June 2013
Handling editor: Kristina Lindström

Keywords:
Allelochemical compounds
Arbuscular mycorrhizal fungi (AMF)
Natural leachates
Plant invasion
Plantago lanceolata

A B S T R A C T

The invasion success of the leguminous tree *Acacia dealbata* Link has been related to the release of novel chemical compounds that affect both native plant performance and native soil bacterial communities. However, the allelopathic effect of *A. dealbata* on arbuscular mycorrhizal fungi (AMF) has not been explored. We used natural leachates from invasive *A. dealbata* and native soils to assess the bioactivity of these chemicals on AMF colonization of *Plantago lanceolata* in native soils. The highly mycorrhizal *P. lanceolata* was used as a model-test species to estimate the infectivity of AM fungi. *Acacia* leachates did not affect mycorrhizal colonization in any of the native soils studied. Either the leachates released by *A. dealbata* do not have an antifungal effect or the analyzed soils contained AMF species resistant to those allelochemicals. Our results appeal for further research to elucidate the role of native AMF in the invasion process of *A. dealbata*.

1. Introduction

The “novel weapons hypothesis” postulates that the success of exotic plants might be related to the release of allelochemical compounds that are novel to native species [1], since exotic and native species have not developed mutual tolerance in the course of a joint evolutionary process [2,3]. Although this theory was originally proposed for plant species, exotic plants can also modify microbial communities by releasing novel chemical compounds into the soil [4,5]. Some invasive plant species can lead to the disruption of native mycorrhizal fungal communities [6,7]. For example, allelochemicals released by invasive plants reduce AMF abundance, growth and colonization, and spore germination in invaded sites [6,8–11]. Mycorrhizal fungi form symbiosis with 90% of plant species, which are dependent on this association for growth and survival [12]. Changes in native mycorrhizal communities by invasive exotic species usually have negative effects on native plant species leading to a rapid degradation of the invaded ecosystem [6–8,13,14].

Acacia dealbata Link is a leguminous tree native to Australia that has become an aggressive invader around the world [15]. Allelopathy has been invoked to partially explain the invasion success of *A. dealbata* since naturally-released chemical compounds of this invasive species were shown to interfere with European species and favor its own seeds [16]. Seed germination, seedling growth, net photosynthetic rate, respiration rate and growth of several plant species are all affected by leachates from *A. dealbata* [17–20]. In addition, the genetic structure and diversity of soil microbial communities in native shrublands and grasslands and the functional diversity of the soil bacterial community from native pine forests can also be altered by natural leachates of this Australian species [5,21].

The present study aims to analyze the potential allelopathic effect of this invader on the infectivity of arbuscular mycorrhizal fungi (AMF). Our hypothesis was that *A. dealbata* releases allelochemicals that lead to a decrease of AMF colonization in native soils.

2. Material and methods

2.1. Collection of natural leachates

The allelopathic effect of *A. dealbata* was assessed by collecting natural leachates that represent natural concentrations of chemical

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http://dx.doi.org/10.1016/j.ejsobi.2013.06.003

Table 1
Locations and characteristics of the native ecosystems where soil collection was done.

Ecosystem	Coordinates	Site	Soil type
Quercus forest	40°12′50.34″N 8°23′58.56″W	Coimbra, Portugal	Lithosol
Pinus pinaster open forest	40°5′39.01″N 8°14′32.99″W	Lousã, Portugal	Lithosol
Shrubland of *Erica* and *Ulex* spp	42° 6′3.12″N 8°38′32.73″W	Tui, Spain	Umbrisol
Grassland	40°13′7.80″N 8°22′26.86″W	Coimbra, Portugal	Cambisol

compounds, a requirement to be able to interpret allelopathy within an ecological context [13].

Natural leachates collection of *A. dealbata* was conducted in Coimbra, Portugal (40°12′30.50″N 8°24′4.21″W) during the rainy season in February 2011. Time collection was selected to coincide with the flowering stage of *A. dealbata*, since this is the peak of production and release of allelochemicals for this species [18,19]. Detailed description of leachates collection can be found in Lorenzo et al. [5]. Distilled water was used as a control. The pH was 6.25 and 5.70 for acacia leachate and distilled water respectively. The ionic concentration of naturally-collected *Acacia* leachates is below the toxic threshold for phytotoxicity [18,19].

2.2. Soil collection

Soil was collected in the last week of April 2012 from four different non-invaded locations (Table 1). Six samples of the top soil layer (0.25 m^2 and 10 cm deep) were randomly collected in each site. Soil samples from the same origin were pooled, stones and roots were manually removed and soil samples were stored in open bags at room temperature. The experiment was done within two weeks after soil collection.

2.3. Experimental setup

The experimental design consisted of 4 soil types (*Quercus* forest, *Pinus* pine forest, shrubland, grassland) × 2 leachate types (natural leachate from the acacia stand, distilled water). Twenty pots were filled with 100 ml of field soil from each site. Ten seeds of *Plantago lanceolata* L., a model species in mycorrhizal studies, were sown in each pot and subsequently thinned to obtain one seedling per pot. Pots were placed outdoors at the Botanical garden of University of Coimbra (40°12′22.94″N 8°25′28.66″W) for nine weeks. Pots were watered with 10 ml of either *A. dealbata* leachates or distilled water as needed. Treatments were replicated 10 times. At the end of the experiment, roots were cleaned, dried at 60 °C for 48 h and subsequently used for determining mycorrhizal colonization.

2.4. Assessment of AMF root colonization

Dried roots of each seedling were rehydrated in distilled water, chopped into 1 cm long fragments and stained according to Walker [22]. Mycorrhizal colonization was assessed using a modified grid-line intersection method [23]. For each sample, occurrence of hyphae, vesicles and arbuscules was recorded at least in 100 intersections points. Vesicles were very rare and values of hyphae and vesicles were added up for data analysis.

2.5. Statistical analysis

We used a one-way ANOVA for each site to test whether leachate type has an effect on the root colonization of *P. lanceolata* by AMF. Verification of normality and homogeneity of variance were assessed by Kolmogorov–Smirnoff test and Levene test respectively. The level of significance was fixed at $P \leq 0.05$. Statistical analyses were performed using SPSS v.19.0 for Windows.

3. Results and discussion

No effect of the *A. dealbata* leachates on the root colonization by arbuscular mycorrhizal fungi (AMF) was detected in any of the studied soils (Fig. 1). The percentage of *P. lanceolata* AMF root infection was around 90% in all studied soils (Fig. 1). *A. dealbata* leachates did not affect either the abundance of arbuscules. The mean values of arbuscules colonization in distilled water and *A. dealbata* leachates were respectively 77.31 ± 1.83 and 73.14 ± 0.84 in the soil from *Pinus* forest, 84.06 ± 3.41 and 83.02 ± 2.97 in the soil from *Quercus* forest, 87.17 ± 2.13 and 85.81 ± 2.20 in the grassland soil, and 93.06 ± 1.55 and 91.01 ± 1.04 in the shrubland. These results did not support our hypothesis that naturally-released allelopathic compounds of *A. dealbata* modify the colonization of *P. lanceolata* by native AMF. Our results did not agree with previous works testing the effect of novel chemicals of other aggressive invaders on AMF that found strong negative allelopathic effects e.g. [6,8]. Therefore, we show that mycorrhizal degradation is not a universal rule for plant invasion. These results suggest that either the leachates released by *A. dealbata* do not have an antifungal effect or that the analyzed soils contained AMF species resistant to those allelochemicals. Using soils from four different ecosystems ensures that the analyzed AMF communities are different [24], and therefore, resistance of the AMF from the four different soils is more unlikely than a general absence of allelopathic effect on AMF. However, this study did not analyze the

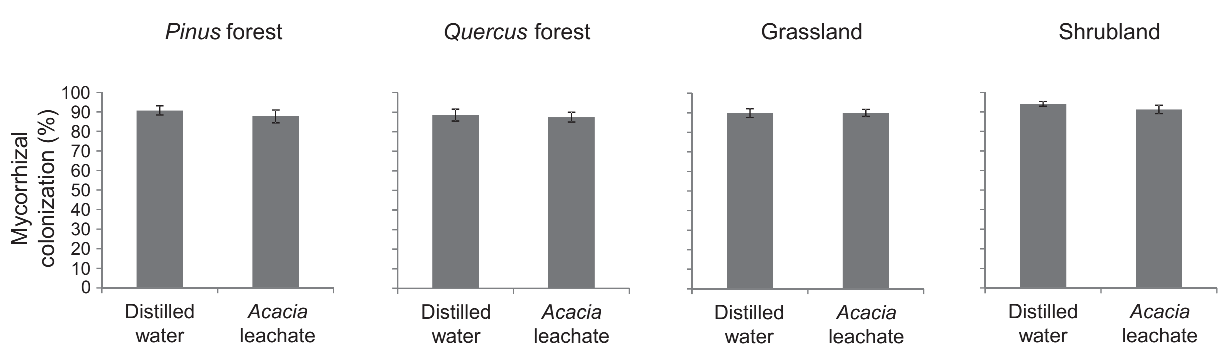

Fig. 1. Percentage of arbuscular mycorrhizal fungi (AMF) root colonization in soils from *Pinus* and *Quercus* forests, grassland and shrubland in response to the application of naturally-released allelochemicals of *Acacia dealbata*. Data are mean ± SE, $n = 10$. No significant differences were found (One-way ANOVA, $P \geq 0.05$).

composition of the AMF colonizing plant roots and we cannot discard changes in the AMF community structure. On the other hand, allelochemicals can be degraded by microbial activity reducing its toxicity [4,25–27] and this could be an alternative explanation for the obtained results.

In conclusion, allelopathic compounds released in field conditions by the invasive *A. dealbata* do not seem to affect native AMF colonization of *P. lanceolata*. However, these results do not imply that AMF are not involved in the invasion by *A. dealbata*. Recent research has showed that the insensitivity of mycorrhizal fungi to allelochemicals may enhance their allelopathic effect for native plants through the transport of these chemicals through common mycorrhizal networks [25]. On the other hand, non-native plants may benefit of mycorrhizal associations in invaded areas [28]. As an example, the growth and development of the invader *Ambrosia artemisiifolia* is increased by the presence of AMF [29]. AMF can also alter the outcome of competitive interactions between invasive and native species. Invasive species such as *Centaurea maculosa* and *Ardisia crenata* benefit from the presence of AMF when competing with native plants through nutrient sharing via the mycorrhizal network [30,31]. Since *A. dealbata* can form mycorrhiza in invaded sites (personal observation), it might be taking advantage of the native AMF communities to colonize and invade ecosystems in new geographical areas.

Acknowledgments

This work was supported by the project MUTUALNET (PTDC/BIA BEC/103507/2008), from the European Union and the Portuguese Foundation for Science and Technology and by post-doctoral research grant from the Spanish Ministerio de Educación (Programa Nacional de Movilidad de Recursos Humanos del Plan Nacional de I-D+i 2008–2011, Ref. EX2010-1169) awarded to Paula Lorenzo.

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