Arbuscular mycorrhizal colonization in black poplar roots after defoliation by a non-native and a native insect

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A major goal in ecology is to understand how interactions among organisms influence ecosystem services. This work compares the effects of two Lepidoptera defoliators, one non-native (Hyphantria cunea) and one native (Lymantria dispar) to Europe, on the colonization of black poplar (the Populus nigra clone “Jean Poullet”) roots by an arbuscular mycorrhizal (AM) symbiotic fungus (Funneliformis mosseae) in a pot experiment. The effects of defoliation have also been assessed on the expression of fungal and plant genes playing a role during symbiosis. Both control and defoliated poplars have shown a low level of mycorrhization. Additionally, neither the non-native nor the native insect seem to strongly affect the AM colonization, at least at the time of observation (eight days from the end of the defoliation). Concerning the gene expression analysis, our results suggest that defoliation does not influence neither the expression of genes coding for a fungal and a plant phosphate transporter nor that of a gene coding for a fungal ATPase, and that there were no differences between defoliation carried out by the non-native and the native insect.

Keywords: Exotic, Defoliators, Arbuscular Mycorrhizal Symbiosis, qPCR, Poplar, Gene Expression

Introduction
Terrestrial ecosystems can be roughly divided into above- and below-ground, linked together by plants (Warde 2002). A major goal in ecology is to understand how interactions among organisms influence ecosystem processes (Bezemer & Van Dam 2005). Invasive organisms – i.e. non-native organisms, which threaten ecosystems, habitat or indigenous species – are a major element of global change and are contributing to biodiversity loss, ecosystem degradation and impairment of ecosystem services worldwide (Baker et al. 2005).

More than 90% of plants form root interactions with arbuscular fungi that provide the host plant with water and mineral nutrients and can lead to an improved tolerance to biotic and abiotic stresses (e.g., salinity, drought, heavy metals), in exchange for photosynthates (Smith & Read 2008). In particular, arbuscular mycorrhizal (AM) symbiosis involves fungi belonging to Glomerales (Schüssler et al. 2001) and the majority of land plants, including both important crop species, such as tomato, wheat, rice, maize, soybean, and forest tree species, such as poplars (Populus spp. – Tisserant et al. 2012). The root colonization process involves both epidermal and cortical cells, where the fungus develops inter- and intra-cellular hyphae, coils, and arbuscules (Genre & Bonfante 2005). Despite the improved mineral nutrition, AM fungi cause a significant carbon cost for the host plant, allocating up to 20% of the photosynthates (Saravési et al. 2014). AM fungi are obligate biotrophs and they are thus not able to survive long periods without the host plant (Bécard et al. 2004). Enzymes for saprotrophic behavior are lacking in the genome of the AM fungus Rhizophagus irregularis, which has been recently sequenced (Tisserant et al. 2013). Over long-term, leaf herbivory (clipping) may eventually result in reduced carbon accessibility for the host plant and fungal partners (Barto & Rillig 2010, Saravési et al. 2014), although, in spite of the re-growth of the above-ground tissue, carbon allocation to roots often increases immediately after defoliation, while allocation to shoots decreases (Dyer et al. 1991, Holland et al. 1996). Although the impact of leaf herbivory on colonization by mycorrhizal fungi has mainly been reported as negative, sometimes even positive or nil effects on fungal symbiosis have been detected (Gehring & Whitham 1994, 2002, Cullings et al. 2001, Eom et al. 2001, Hokka et al. 2004, Kula et al. 2005, Pietikäinen et al. 2005, Walling & Zabiniski 2006, Gehring & Bennett 2009, Saravési et al. 2014, Trocha et al. 2015). It has been reported that the variability in responses might depend on the type and extent of defoliation, the duration of the experiment, the mycorrhizal type or plant species considered, the availability of soil nutrients or other not yet known factors (Gehring &
Whitham 2002, Gehring & Bennett 2009). Barto & Rillig (2010) conducted a meta-analysis study, focusing on the sensitivity of AM and ectomycorrhizal (ECM) colonization of roots to the removal of leaves or shoots by herbivory or clipping. Meta-analysis results showed that the carbon-limitation hypothesis of reduced mycorrhizal colonization following removal of above-ground biomass was generally not supported, and that herbivory (or clipping) did not reduce mycorrhizal colonization by biologically meaningful levels in many types of plants (Barto & Rillig 2010). More recently, Saravesi et al. (2014) reported a different effect of host plant defoliation on root AM and dark sepiate endophyte (DSE) fungal colonization. In particular, negative impacts on AM colonization and a slightly positive impact on DSE colonization were observed, suggesting divergent ecological roles of the two fungal associates in the plant carbon economy.

The increasing threat of invasive species, among which are several defoliators, raises a question about their possible impact on the components of native ecosystems (Kenis et al. 2009). In Europe, Hyphantria cunea Drury, a moth native to North America, became invasive in the 1940s (Warren & Tadic 1970). Being a polyphagous defoliator, it feeds on hundreds of host plants, including black poplars. P. nigra L. is also a host plant for native defoliators, such as Lymantria dispar L. in a scenario where losses of above-ground biomass might impact mycorrhizal response as previously reported (Gehring & Whitham 2002, Gehring & Bennett 2009), the issue is whether invasive defoliators could play a role in affecting native ecosystem components such as AM fungi.

In this work we compare the effects of two Lepidoptera defoliators, a species non-native to Europe, H. cunea, and a native species, L. dispar, on black poplar (the P. nigra L. clone “Jean Pourtet”) roots colonized by the AM fungus Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler 2010. In particular, we assessed the AM fungal colonization in the black poplar roots and the expression of fungal genes coding for a fungal amino acid permease (GmosAAP1 – Cappellazzo et al. 2008), a phosphate transporter (GmosPT – Balestrini et al. 2007) and two different H’-ATPases (GmHA5, GmPMA1 – Requena et al. 2003). The fungal reference gene was the elongation factor (GmEF1) – Cappellazzo et al. 2008). Concerning the plant, an elongation factor 1, beta subunit (P00EF1) was used as a reference gene (Regler et al. 2009), in addition to a phosphate transporter (P00PT) for which the primers were designed using the web-interface program Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). To this aim, the mycorrhizal-inducible Solanum lycopersicum phosphate transporter 4 (LePT4 – accession AAY97730.2) was used to search the homologous in the genome of P. trichoarpa (http://phytosome.jgi.doe.gov/pj2/portal.html?info=Org_Ptrichocarpa – Tuskan et al. 2006). Among the P. trichoarpa homologous sequences identified using the tomato LePT4 sequence, the Potri015G02800.1 was the best hit. The poplar and tomato aminoacid sequences showed 76% of identity and 98% of coverage with 0.0 of E-value. In addition, the poplar aminoacid sequence showed 74% of identity and 98% of coverage with 0.0 of E-value with the high affinity inorganic phosphate transporter of Medicago truncatula (accession XP_013466381.1), which corresponds to the mycorrhiza-inducible MtPT4 (accession AAM76744.1).

The primers used in this work are listed in Tab. 1. Fungal primers were tested on poplar DNA in order to verify the absence of cross-amplification. The primers for PopPT were tested in silico to verify the 180 °C for 3h. For one month, they were watered twice a week with tap water in order to obtain shoots and roots. The DNA was extracted from about 100 mg of roots using the “pine-tree method” (Chang et al. 1993). After extraction, RNA was cleaned of DNA, using Promega DNase (RQI RNase-Free DNase®, Promega Corp., Madison, WI, USA), and then measured using a NanoDrop® (ThermoScientific, Hudson, NH, USA). The absence of genomic DNA was verified through one-step retro-transcription PCR (One-Step RT-PCR, Qiagen) with primers (GmosEF, GmEF1) previously designed for the F. mosseae elongation factor reference gene (Cappellazzo et al. 2008). Briefly, the same extracted RNA was used as template for retro-transcription and for PCR amplification or for PCR amplification only (RT-reactions). The absence of signal after PCR amplification without retrotranscription was regarded as absence of DNA. Total cDNA for each sample was used to synthesize the cDNA, according to the SuperScript II Reverse Transcription® (Invitrogen, Carlsbad, CA, USA) procedure using random primers. Selected genes and primers

The fungal genes selected for the analysis were an amino acid permease (GmosAAP1 – Cappellazzo et al. 2008), a phosphate transporter (GmosPT – Balestrini et al. 2007) and two different H’-ATPases (GmHA5, GmPMA1 – Requena et al. 2003). Among the P. trichoarpa homologous sequences identified using the tomato LePT4 sequence, the Potri015G02800.1 was the best hit. The poplar and tomato aminoacid sequences showed 76% of identity and 98% of coverage with 0.0 of E-value. In addition, the poplar aminoacid sequence showed 74% of identity and 98% of coverage with 0.0 of E-value with the high affinity inorganic phosphate transporter of Medicago truncatula (accession XP_013466381.1), which corresponds to the mycorrhiza-inducible MtPT4 (accession AAM76744.1).

The primers used in this work are listed in Tab. 1. Fungal primers were tested on poplar DNA in order to verify the absence of cross-amplification. The primers for PopPT were tested in silico to verify the
amplification on the target gene. In addition, PCR fragment has been sequenced at BMR Genomics S.r.L. (Padua, Italy).

Relative fungal DNA abundance

To evaluate the relative fungal abundance in poplar roots, quantitative PCR (qPCR) was carried out with Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Each PCR reaction was conducted on a total volume of 10 μl, containing 1 μl DNA (4.5 ng μl⁻¹), 5 μl SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories), 3.4 μl of water and 0.3 μl of each primer (10 μM), using a 96 well plate. The reference genes for the fungus (elongation factor – Cappellazzo et al. 2008) and for the plant (elongation factor 1 subunit beta – Regier et al. 2009) are listed in Tab. 1. The following PCR program, which includes the calculation of a melting curve, was used: 95 °C for 30 s, 45 cycles of 95 °C for 30 s, 45 cycles of temperature for 30 s, ramp from 65 to 93 °C with a temperature increment of 0.5 °C and a read plate every 2 s. All the reactions were performed for three biological and three technical replicates. The baseline range and Ct values were automatically calculated using the Bio-Rad CFX Manager software. The fungal reference gene expression was normalized to that of the plant reference gene by subtracting the Ct value of all the genes were normalized to the Ct value of the reference. Candidate gene expression was normalized to that of the reference gene by subtracting the Ct value of the candidate gene from the Ct value of the reference gene without efficiency correction, from equation 2

\[
\Delta C_t = C_t_{\text{sample}} - C_t_{\text{control}}
\]

It has been reported that herbivores, such as insects, could have contradictory effects on the mycorrhization (Barto & Rilloner 2009), (Livak & Schmittgen 2001), where ΔCt represents the ΔCt sample - ΔCt control (control plants). The Kruskal Wallis test was carried out using Past version 3.07, with a 0.05 cut-off level of significance.

Gene expression analysis

Gene expression analyses on plant and fungal genes were performed by quantitative real-time (RT-qPCR) on the cDNAs produced starting from the extracted RNAs, using the same machine, the same mix and the same PCR program described above. The primers used are listed in Tab. 1. In order to compare the data from different PCR runs or cDNA samples, the Ct values of all the genes were normalized to the Ct value of the reference. The extent of defoliation were evaluated at the level of mycorrhizal colonization and on the expression of specific genes, which play a role in symbiosis functioning.

All poplar leaves were defoliated similarly by the two insects, resulting in a 50% defoliation in the partial defoliation experiment and a 100% defoliation in the total defoliation experiment.

Extent of colonization

AM fungus colonization occurred in both control and defoliated poplars, although it never reached very high levels, as previously reported on the same poplar clone (Lingua et al. 2008) and on P. alba (Lingua et al. 2012). The colonization was very patchy with some fragments very highly colonized and the most uncolonized. However, morphological observations in the colonized fragments allowed to identify the presence of the typical structures of the symbiosis (such as inter- and intracellular

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene Function</th>
<th>Reference</th>
<th>Sequence</th>
<th>Ta (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GmosPT For</td>
<td>Pi transporter</td>
<td>Balestrini et al. 2007</td>
<td>ACTGTTGGCGCTTAGTGCTTG</td>
<td>60</td>
</tr>
<tr>
<td>GmosPT Rev</td>
<td></td>
<td></td>
<td>CAGCCGACTTGTTGATTGCG</td>
<td></td>
</tr>
<tr>
<td>GmPMA1F3</td>
<td>H⁺-ATPase</td>
<td>Requena et al. 2003</td>
<td>CCAAGTCAAGAGTTCCCAAG</td>
<td>60</td>
</tr>
<tr>
<td>GmPMA1R1</td>
<td></td>
<td></td>
<td>CGTTCGCTGTGATATCG</td>
<td></td>
</tr>
<tr>
<td>GmHAsup1</td>
<td></td>
<td></td>
<td>AAATATGTCCTGGAGCGAGG</td>
<td></td>
</tr>
<tr>
<td>GmHAsup3</td>
<td></td>
<td></td>
<td>TTGAATGCTGTCCTTTTGGC</td>
<td></td>
</tr>
<tr>
<td>Gmofst1f</td>
<td>Elongation factor</td>
<td>Cappellazzo et al. 2008</td>
<td>GCAGAAGCTGAGCTGTGAT</td>
<td>63</td>
</tr>
<tr>
<td>Gmofst2r</td>
<td></td>
<td></td>
<td>ACCAGTTCGGCGCAGAATAA</td>
<td></td>
</tr>
<tr>
<td>GmosA1P1</td>
<td>Amino acid permease</td>
<td></td>
<td>TACTCTCCACCAGGATACG</td>
<td>54</td>
</tr>
<tr>
<td>GmosA1P2</td>
<td></td>
<td></td>
<td>CCGATGATGATGATGCGGAT</td>
<td></td>
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<tr>
<td>PopPT4or</td>
<td>Poplar phosphate transporter</td>
<td>This work</td>
<td>TACCAACCTACGCCGGAT</td>
<td>60</td>
</tr>
<tr>
<td>PopPT4rev</td>
<td></td>
<td></td>
<td>CGGTTCCTGCGGCATCTC</td>
<td></td>
</tr>
<tr>
<td>EF1 F</td>
<td>Poplar elongation factor 1,</td>
<td>Regier et al. 2009</td>
<td>AAGCCATGGGATGAGAAC</td>
<td>60</td>
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<tr>
<td>EF1 R</td>
<td>beta subunit</td>
<td></td>
<td>ACTGGAGCAAATTTTGATG</td>
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</tr>
</tbody>
</table>

### Effect of defoliation on AM colonization

**Tab. 2 – Relative DNA quantities of F. mosseae obtained from the qPCR assays on the roots of P. nigra.**

<table>
<thead>
<tr>
<th>Biological replicate</th>
<th>Ct Gmosfet</th>
<th>Ct PopEF1</th>
<th>ΔCt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plant_C8</td>
<td>32.91</td>
<td>24.58</td>
<td>8.34</td>
</tr>
<tr>
<td>Control plant_C9</td>
<td>30.20</td>
<td>24.31</td>
<td>5.89</td>
</tr>
<tr>
<td>Control plant_C21</td>
<td>30.10</td>
<td>23.55</td>
<td>6.55</td>
</tr>
<tr>
<td>Hypanthria cunea_100_1</td>
<td>31.46</td>
<td>24.25</td>
<td>7.22</td>
</tr>
<tr>
<td>Hypanthria cunea_100_2</td>
<td>28.01</td>
<td>23.94</td>
<td>4.07</td>
</tr>
<tr>
<td>Hypanthria cunea_100_28</td>
<td>28.71</td>
<td>24.23</td>
<td>4.48</td>
</tr>
<tr>
<td>Hypanthria cunea_50_7</td>
<td>30.31</td>
<td>24.91</td>
<td>5.39</td>
</tr>
<tr>
<td>Hypanthria cunea_50_12</td>
<td>30.36</td>
<td>23.86</td>
<td>6.50</td>
</tr>
<tr>
<td>Hypanthria cunea_50_26</td>
<td>36.15</td>
<td>23.42</td>
<td>12.73</td>
</tr>
<tr>
<td>Lymantria dispar_100_5</td>
<td>29.33</td>
<td>25.21</td>
<td>4.13</td>
</tr>
<tr>
<td>Lymantria dispar_100_6</td>
<td>32.17</td>
<td>24.86</td>
<td>7.32</td>
</tr>
<tr>
<td>Lymantria dispar_100_16</td>
<td>31.54</td>
<td>25.28</td>
<td>6.27</td>
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<tr>
<td>Lymantria dispar_50_4</td>
<td>31.63</td>
<td>23.83</td>
<td>7.80</td>
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<tr>
<td>Lymantria dispar_50_6</td>
<td>31.67</td>
<td>24.86</td>
<td>6.81</td>
</tr>
<tr>
<td>Lymantria dispar_50_19</td>
<td>29.87</td>
<td>25.07</td>
<td>4.81</td>
</tr>
</tbody>
</table>
hyphae, coils, vesicles and arbuscules) in the roots of plants, regardless of the thesis (Fig. 1). Here, the low level of colonization, as well as the quality of the root segments after the staining, made morphological quantification difficult, and therefore the presence of the AM fungus has been quantified by calculating the relative fungal DNA abundance (Tab. 2). Quantitative PCR (qPCR) results showed that fungal Cts are higher than plant ones in all the theses, suggesting that fungal DNA is in lower amount than the plant one as previously described (Li et al. 2008). Statistical analysis showed that there was no significant difference in the amount of fungal DNA in the roots of plants from the different theses (p > 0.05).

Gene expression analysis
To verify the effect of defoliation on AM symbiosis functioning, the expression of fungal genes, for which a previous role during symbiosis was reported, has been evaluated (Requena et al. 2003, Balestrini et al. 2007, Cappellazzo et al. 2008, Gomez-Ariza et al. 2009). Due to their role in symbiosis, fungal and plant PT genes are considered as key genes for the symbiosis (Balestrini et al. 2007, Bucher 2007). Together with the expression of fungal genes, we analyzed a poplar PT gene (PopPT), selected on the basis of the homology with a
tomato PT gene (LePT4), which is considered as a marker for a functional symbiosis (Nagy et al. 2005, Xu et al. 2007).

The ΔCt for each biological replicate was shown in Tab. S1 in Supplementary material, coupled with mean and standard deviation (SD). In Tab. S2 (Supplementary material) the Ct values coupled with mean and standard deviation (SD) were shown for each gene and for each technical replicate.

Starting from the fungal genes, GmosAAP1 gene expression was detected in one-step RT-PCR (data not shown), while it was not possible to quantify its transcripts in RT-qPCR. Cappellazzo et al. (2008) demonstrated that this gene is expressed in the extra-radical mycelium, but not in the intraradical one, suggesting a role in the first steps of amino acid achievement and in favoring a direct amino acid uptake from the soil. In poplar-F. mosseae association, it was not possible to quantify its expression by RT-qPCR, while a signal has been detected by one-step RT-PCR. This may suggest that this AM fungus can produce only a small quantity of extra-radical mycelium in our system or that only a little amount of extra-radical mycelium remained attached to the roots after the repotting. Concerning the two H’-ATPases, GmHA5 was expressed in all the five theses and no different expression was assessed in control and defoliated plants (p > 0.05 - Fig. 2b), while GmPMA1 transcripts were detected only in one replicate of the defoliated samples using one-step RT-PCR (data not shown), and not in RT-qPCR. Looking at the data previously reported by Requena et al. (2003), GmHA5 showed a very low expression during non-symbiotic growth and a highly induced expression in mycorrhizal roots and in extra-radical hyphae. GmPMA1 was on the contrary more expressed in the pre-symbiosis and less in the late stages of symbiosis, in agreement with our results. H’-ATPases play a role in the establishment of an electrochemical gradient, necessary for the transport of nutrients across the plasma membrane in both fungi and plants, but each isoform could be engaged at different developmental stages, probably in function of the different necessities in the symbiosis (Requena et al. 2003).

In this study, no significant differences in GmosPT expression were observed between the control and defoliated samples (p > 0.05 - Fig. 2a), suggesting that the feeding activity of insects has no influence on the phosphate (Pi) transport by the fungus. A role and a control action in Pi transfer by GmosPT was suggested and its expression was reported both in extra- and intra-radical mycelium (Benedetto et al. 2005). Our results suggest that defoliation does not influence the expression of this fungal gene and that there are no differences between defoliation carried out by the non-native or the native insect considered, at least at the sampling time (eight days after the end of the defoliation). As regards the plant, the expression of the selected poplar PT gene was not affected by the defoliation in any of the theses (Fig. 2c). In tomato mycorrhizal roots LePT4 transcripts have been specifically detected in arbuscule-containing cells together with other tomato PT genes and GmosPT (Balestrini et al. 2007, Gomez-Ariza et al. 2009), suggesting that in arbusculated cells the fungus can compete with the plant for the Pi uptake. Taken together, the results on PT genes, which are considered as markers of a functional symbiosis, confirm the quantitative results, showing no differences in the mycorrhization among the samples.

Additionally, neither the non-native nor the native insect seem to strongly affect the AM colonization, at least at the time of observation.

Previous studies on the impact of defoliation on roots colonization by AM fungi reported controversial results. In Lotus tenuis, the reduction in the photosynthetic capacity by defoliation did not change the root length colonization by AM fungi and the proportion of root colonized by arbuscules, while it decreased vesicular colonization. García & Mendoza (2012) suggested that the strategy of the AM fungus consisted in investing more of the C resources to preferentially maintain the arbuscules and the inoculum density in the soil by exporting C compounds to retain extra-radical structures such as spores and the hyphal network. In woody species, the extensive below-ground carbon reserves could limit the effects of defoliation on mycorrhizal colonization, even after the loss of large amounts of above-ground biomass (Kosola et al. 2004). In particular, the results of a field experiment with the hybrid poplar Populus x canadensis Moench "Eugenei" showed that neither ECM nor AM colonization were affected by L. dispar defoliation during two years. Barto & Rillig (2010), through a meta-analysis of 99 experiments from 33 publications, suggested that, in contrast with the carbon-limitation hypothesis, herbivory did not reduce mycorrhizal colonization by biologically significant levels in the majority of plants, including perennial grasses and forbs. Recently, Trocha et al. (2015) have not found changes in the composition of ECM communities of Fagus sylvatica and Pinus sylvestris after damages, including defoliation. On the contrary, Markkola et al. (2004) and Saravesi et al. (2008) found changes in the ECM composition when Betula pubescens or P. sylvestris were defoliated, suggesting that high-biomass ECM fungal species need great quantities of carbon because they found a reduction of these fungi after defoliation.
Conclusions

Although the poplar roots showed a low level of AM fungal colonization in all the treatments, our results suggest that neither the non-native nor the native insect considered seem to strongly affect the AM colonization, at least in our conditions and sampling time. Two levels of defoliation (total and partial) were considered and the impact of the defoliators on the colonization by an AM fungus was investigated, showing the expression of fungal and plant genes putatively involved in symbiosis functioning. No significant differences were found between the treatments, and no significant differences compared to the undefoliated ones. Despite several studies simulated herbivory effects on plants using mechanical defoliation or included mechanically defoliated control plants, this approach is deemed appropriate when studying simple biotic interactions, but not necessarily for complex interactions including different trophic levels (Hjältén 2004, Lehtilä & Boalt 2004), as the ones investigated. Although most of the papers published on AM symbiosis report open field experiments, pot experiments in semi-controlled conditions, like those performed in this work, have allowed to study some of the mechanisms involved in plant-AM fungi interaction under defoliation conditions. However, it cannot be excluded that a longer time after defoliation would be needed to affect fungal colonization. In addition, an analysis of other fungal genes (such as a hoxene transporter gene) could be useful to obtain a more complete picture, but unfortunately the F. mosseae genome has not been sequenced and the sequences available are still limited. Interestingly, differential impact of seasonal defoliation on root fungal symbionts has also been reported (Saravadi et al. 2008). It could be interesting to compare the data obtained in this work employing other defoliators, or to study the AM symbiosis in defoliated poplars directly in nature. As suggested by Saravadi et al. (2008), different herbivore organisms, which have dissimilar feeding regimes during the growing season, could have diverse effects on the composition or condition of fungal symbionts. Data presented in this study improve the knowledge on differential effect that invasive organisms may have compared to native ones on plant symbionts, an issue recently reported for plant pathogens (Sillo et al. 2015), but rarely investigated for defoliators.

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References


Effect of defoliation on AM colonization


Supplementary Material

Tab. S1 - Relative expression of three different genes in the 15 used biological replicates.

Tab. S2 - Ct values of each technical replic- ate of four different genes in the 15 used biological replicates.

Link: Zampieri_1911@supplpdf1