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ESTIMATION OF THE ECTO- AND ENDOMYCORRHIZAL COLONISATION OF THE BLACK POPLAR - *POPULUS NIGRA*- OF AIT ZIKKI (KABYLIA, ALGERIA)

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ABSTRACT

Very little work has been done on the mycorrhizal state of the black poplar in Algeria. Therefore, the purpose of our investigation is to make an inventory of the mycorrhizal procession of the black poplar in the region of Kabylia (Algeria). We were interested in seeing the influence of age, position of trees compared to a river and the influence of soil factors on the relative rates of the two types of mycorrhizae. Root samples were collected from four trees of different ages in March 2015. The trees were in different positions in relation to a watercourse. A quantification of the different types of mycorrhizae was performed by means of the method suggested by Giovanetti and Mosse (1980). Physicochemical analyzes were performed on the soils collected around the trees. The results show some morphotypic wealth in ectomycorrhizal and some diversity of mycorrhizal structures. The quantification revealed that endomycorrhizal colonization was superior to ectomycorrhizal colonization, whatever the age of the trees and their position in relation to the watercourse. The youngest tree showed the highest rate in AM. Indeed, we noted negative correlations between the age variable and colonization by AMs. These results are essentially due to the physicochemical factors of the soil.

KEYWORDS: Age, Endo- and Ectomycorrhizal, Soil Composition, *Populus nigra*.

INTRODUCTION

The black poplar (*Populus nigra*) is a very old natural plant species of the Mediterranean basin. Formerly considered as an important part of the agricultural landscape, it now ranks among the stress-tolerant species and used in the reforestation of post agricultural land, soil degraded by industry and for the production of bioenergies (Sebastiani et al. 2004; Yin et al, 2005; Monclus et al, 2006). It is a fast-growing species, and its culture facility makes of it the most appropriate essence in intensive cultivation for biomass production (Labiod, 2007). Being a growing wild in northern Algeria, the black poplar is present on mesophilic xerophilic trays and along steep-sided water streams. It is a rare species encountered at the edge of wadis, the mount of Tlemcen, Kabylia and the Aurès mountain range (Mate 1997). The poplar is also used in Algeria as a seed-bearer species. But now some stands are dying out and their future seems seriously threatened. The poplar is among the rare species that can develop two types of mycorrhizae: the ectomycorrhizae (EcM) and endomycorrhizae (AM) (Guard, 2003).

The crucial role of mycorrhizae in plant nutrition and tolerance to abiotic and biotic factors is well established (Lodge 1989; Gehring et al, 2006). Several studies (Vozzo and Hacskalyo, 1974; Lodge, 1989; Brundrett et al, 1990; Neville et al 2002; Khasa et al, 2002; Welc 2004; Gehring et al, 2006) have allowed the observation of colonization

variable rates by EcM and AM of the roots of a large number of poplar species and their hybrids.

Various factors influence the colonization by the CHMS and AMs, such as the age of the tree (Dominik, 1958; Malajczuk Gardner, 1988; Paul and Clark, 1996; Van Der Heijden et al, 1999 ; Chen et al, 2000; Gonçalves and Swifts - Loução 1996), the potential of fungal inoculum (Van Der Heijden and Vosatka 1999), the litter accumulation (Conn and Dighton, 2000), the availability of phosphorus and nitrogen (Baum et Makeschin, 2000), and soil moisture (Truszkowska, 1953 ; Lodge 1989 ; Neville et al, 2002. Gehring et al, 2006). Little work has been done on the mycorrhizal state of the black poplar *Populus nigra*. Accordingly, the main aim of our investigation is to make an inventory of the mycorrhizal procession of the black poplar in the region of Kabylia (Algeria) and see the effect of age and position of the tree in relation to waterways and the effect of soil physicochemical properties on the relative rates of the two types of mycorrhizae in this species.

MATERIALS AND METHODS

Sampling Site

The study site (geographic coordinates: 36° 34' 0" N and 4° 31' 0" E in DMS) is located in Ait Zikki, some forty km from the capital of the Wilaya of Tizi Ouzou (Algeria). The removal of roots was performed on March 2015 characterized by rainfall P= 93 mm and average temperature t=10.8°C, and it involved four *Populus nigra* trees. Four samples were taken around each tree at 1.50m from the trunk and at a depth of 20cm. The trees are located at variable distance from each other and also relative to the streams. They are designated by numbers from 1 to 4. Trees 1 and 4 are spaced from each other by about 400m and are removed from the streams. Trees 2 and 3 are moved away from one another of about 200m and are very close to the watercourse. Trees 1 and 4 were far from the watercourse whereas trees 2 and 3 were on the edge of a watercourse. An auger was used to determine the age of the trees. The vegetation accompanying the *Populus nigra* trees consists of holm oak (*Quercus ilex* L.) with a shrub layer made of cherry (*Prunus avium*), elm (*Ulmus communis*), broom (*Calycotome spinosa*), ash (*Fraxinus angustifolia*), dyss (*Ampelodesma mauritanica*) and blackberry (*Rubus fruticosus*).

Inventory of Mycorrhizae

The ectomycorrhizae are observed on fresh roots under a binocular microscope then described and classified in different morphotypes according to the abundance of their extra-matrix mycelium, their color, their shape and the presence or absence of cords and rhizomorphs. The endomycorrhizae are described after treatment of the roots using the technique of Phillips and Heymann (1970). The endomycorrhizal structures sought for are: vesicles, intra- and intercellular hyphae, platoons and arbuscules. The observations are made with an optical microscope at magnification 400.

Assessment of Mycorrhizal Colonization

The estimate of mycorrhizal colonization is carried out by the grid-line intersect method advocated by Giovannetti and Mosse (1980). It is expressed as a percentage of length of mycorrhized roots. The morphotypical richness in EcM is defined as the number of morphotypes noted per individual.

Soil Analyses

The soil samples around each tree are subjected to physical and chemical analysis in the laboratory of the

Department of Soil Science at the National School of Agronomy (ENSA) of Algiers. The parameters analyzed are the water pH, the determination of phosphorus, total nitrogen, organic matter and texture by particle size analysis. The results obtained are interpreted by means of the standards suggested by Villeman and Calvet (1986).

Statistical Analyses

The results are subjected to variance analysis (at P = 0.05 level) complemented by the multiple comparison test of average designed by Newman and Kells by means of the Biostat9 software. An analysis of the main components (ACP) is performed using the StatBox 6 software in order to determine the effect of age, proximity to rivers and soil composition on the relative rate of ECMs and AMs.

RESULTS

The trees involved in our study are of different ages. Tree 1 is 50 years old, tree 2 is 73 years old, tree 3 is 60 years old, and finally tree 4 is aged 33.

Physicochemical Analysis of the Soil

The soil analysis of the four trees reveals soils with very alkaline pH, low in nitrogen, very rich in phosphorous and with low organic matter content (Table 1). The texture of the different soils is silty (Table2).

Table 1: Physico-Chemical Data of Soil Taken Around Roots of the Four Trees

	pH water	Total nitrogen %	P Olsen ppm	Organic Material%	Texture
Tree1	6,84	0,020	81,75	1,43	
Tree2	6,89	0,024	84,2	1,75	Silty
Tree3	6,82	0,020	83,7	2,14	
Tree4	7,07	0,030	91,3	2,79	

The Ectomycorrhizae

We observed different morphotypes (Figure 1).

- In tree 1 - aged 50 years, 22 morphotypes were observed, with a dominance of mycorrhizae characterized by the abundance of extra-matrix mycelia.
- In tree 2 - aged 73 years, 15 morphotypes were observed, with a dominance of mycorrhizae characterized by the abundance of extra-matrix mycelia.
- In tree - 3 aged 60 years, 10 morphotypes were observed, all of them with scarce mycelium.
- In tree 4 - aged 33, 12 morphotypes were observed with a dominance of mycorrhizae characterized by the abundance of extra-matrix mycelia.

The quantitative analysis of ectomycorrhizal morphotypes of the different trees highlights the richness in morphotype of the oldest tree, be it at the edge or away from the water stream (Figure 1). The analysis of variance has shown a highly significant difference between the number of morphotypes and the age of the tree (P = 0.0066).

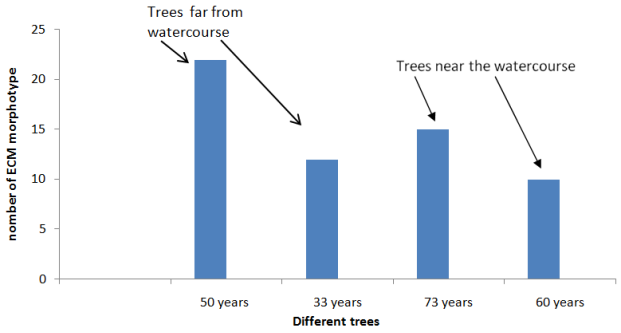

Figure 1: ECM Morphotypic Richness of Populus Nigra (Ait Zikki Station)

The Endomycorrhizae

The observation of the roots revealed the presence of different endomycorrhizal structures, namely: Arum and Paris types of arbuscules, platoons, round and oval intracellular vesicles with or without lipid globules, intracellular and extracellular spores, and hyphae of different siphoned diameters or septa (Table 3).

Table 2: Endomycorrhizal Structures Observed in Roots of Populus Nigra(Ait Zizzi Station)

	Arum Arbuscules	Paris Arbuscules	Roud Vésicles	Oval Vésicles	Peloton	Septa Extracellular Hyphae	Siphoned Extracellular Hyphae
Tree 1	+	-	+	+	+	+	+
Tree 2	+	-	+	+	+	+	+
Tree 3	-	+	+	+	+	+	+
Tree 4	+	-	+	+	+	+	+

+: présent, - : absent

Estimate of Mycorrhizal Colonization

The estimate of mycorrhizal colonization of different trees shows significant differences between endo- and ectomycorrhizal colonization. The length of the endomycorrhized roots seems more important than that of the ectomycorrhized roots (Figure 2). The statistical analysis shows a highly significant difference in colonization for tree 2 (P = 0.0001), tree 3 (P = 0.0002) and tree 4 (P = 0.0152). However, no difference is observed in the case of tree 1.

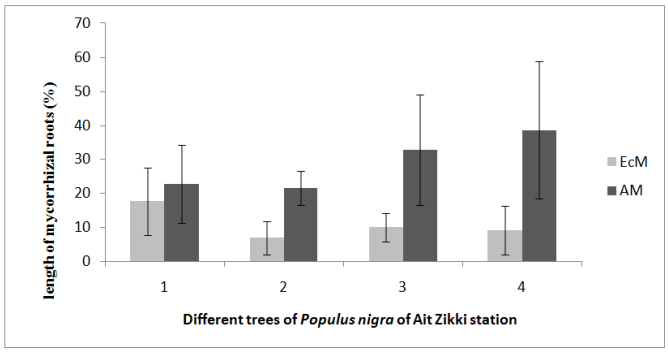

Figure 2: Ectomycorhiza and Endomycorhriza Root Length of Populus Nigra Trees

The biplot distribution of main component analysis (Figure 3) shown in two factorial axes (inertia 89%) shows negative correlations, though not significantly, between the root colonization rate by the CHMS and colonization AMs (R = -0.28, P = 0.05). On the contrary, negative correlations are observed between the age variable and colonization by AMs

(R = -0.71, P = 0.05). This suggests that endomycorrhizal colonization in the young tree is higher. As regards the phosphorus element, the correlation is negative (though not significantly: R = -0.48) with ECMs, and it is positive with the rate of AMs (R = 0.80). As for the nitrogen element, the correlation is negative (though not significantly: R = -0.51) with the ECMs, and it is positive with AMs (though not significantly: R = 0.58). For the position variable in relation to water streams, CPA brings up a positive correlation with both ECMs (R = 0.62) and AMs (though not significantly: R = 0.24).

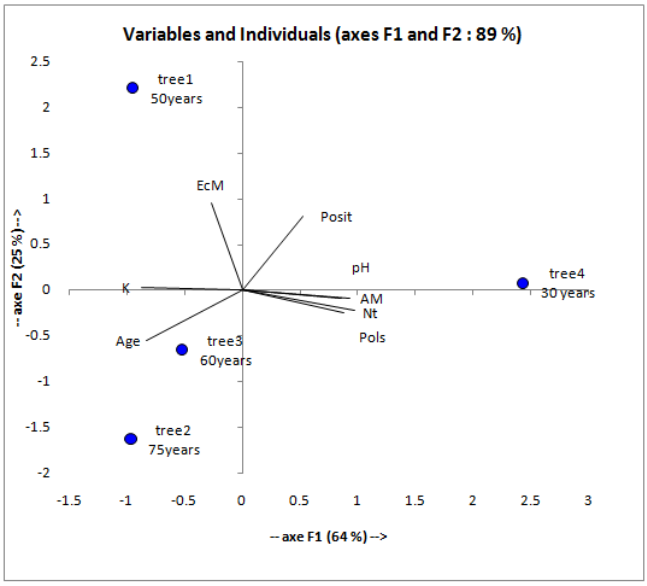

Figure3: Graphic Representation of the Various ACP Variable

DISCUSSIONS

The observation of fresh roots of *Populus nigra* of Ait Zikki has revealed some richness in ectomycorrhizal morphotypes. The most observed morphological type is the one characterized by abundant extra-matrix mycelium. This is in agreement with Gardes (2003) that reports a diversity of ECM communities of the black poplar depending on the habitat and the age of the tree.

Various endomycorrhizal structures have been observed in the roots of *Populus nigra* of Ait Zikki, confirming that the black poplar is a species with a double symbiosis as reported by Lodge (1989) and Lodge and Wentworth (1990)

The quantification of mycorrhization shows the dominance of arbuscular mycorrhizae in all of the four trees, regardless of their age and their position in relation to the watercourse. The most important endomycorrhizal colonization is observed in the youngest tree. This result is in agreement with that of Gardes et al. (2003) who observed more AMs on young poplars. Similarly, Piotrowski et al. (2008) have reported a peak of colonization by AMs in young *Populus* trees.

Ectomycorrhizal colonization is lower than endomycorrhization in all of the four *Populus. nigra* trees. However, Gardes et al. (2003) and Piotrowski et al. (2008), for their part, have noted in the black poplar increased ectomycorrhizal colonization in accordance with the tree age.

Negative correlations between colonization by the ECMs and the availability of nitrogen (R = -0.51, P = 0.05) and the phosphorus element (R = -0.48, P = 0.05) have been noted in the present study. Positive correlations have been also noted between colonization by AMs and the availability of nitrogen (R = 0.58, P = 0.05) and phosphorus (R = 0.80, P =

0.05). According to several authors (Truszkowska, 1953; Lodge, 1989; Baum and Makeschin, 2000; Conn and Dighton, 2000; Neville et al., 2002; Gehring et al., 2006), colonization by the ECMs and AMs is influenced by the physicochemical properties of the soil.

The soil taken around roots of the different trees is very poor in total nitrogen and organic matter. The AMs have thus the ability to effectively absorb mineral nitrogen and amino acids that can be present in the soil (Courty et al., 2010; Smith and Smith, 2011) and transfer it to the plant.

Indeed, the different soils of the study station have neutral pH (between 6.82 and 7.07) that could prevent phosphorus absorption by the root system. This can be assimilated to a phosphorus deficit for the plant. This may also explain, in our case, the high rate of observed AMs, considering that AMs are known to be efficient in the absorption of phosphorus in case of deficit (Read, 1989). It is well known that the phosphorus uptake by roots in alkaline and neutral soils is reduced (Bolan, 1991).

We have also noticed that whatever the position of the trees with respect to the watercourse, endomycorrhizal colonization is dominant. It is worth noting that the collection of roots was conducted in March, a month characterized by an average rainfall of 93 mm, the soil not being dry. The roots of trees 1 and 4 were in moist while those of trees 2 and 3 were in flooded soil because they had grown at the edge of a water stream. High rates of colonization by AMs have been noticed in moist or even flooded soils (Lodge, 1989; Miller, 2000; Entry et al., 2002). An increase of colonization by AMs of roots growing in waterlogged soils at certain times has been observed by Truszkowska (1953) in Alnus Lodge (1989) in *Salix nigra* and by Miller and Bever (1999) in *Panicum hemitomon*.

Older trees recruit more ectomycorrhizal morphotypes. It seems that morphotypic wealth be greater in older trees. Indeed, for the trees near the river, 22 morphotypes have been described in the tree aged 50 years and 12 morphotypes in the tree aged 33 years. Similarly, for the trees near the river, we have described 15 morphotypes in the one aged 73 years and 10 morphotypes in the one aged 60 years.

CONCLUSIONS

The study of mycorrhizal procession of black poplar (*Populus nigra*) of Ait Zikki in the Kabylian region (Algeria) has shown some morphotypic richness in ectomycorrhizae and various endomycorrhizal structures. Endomycorrhizal colonization expressed in percentage of mycorrhized roots has revealed to be more important than ectomycorrhizal colonization, regardless of the age of the tree and whatever the position of the trees in relation to the watercourse. The number of EcM morphotypes, however, increases with age. It seems that the composition of the soil (especially in nitrogen and phosphorus) is at the origin of this difference in colonization by the two types of mycorrhizae. It would be interesting to identify the fungal partners associated with the black poplar which has shown richness of mycorrhizal and ectomycorrhizal fungal assemblages and to follow colonization at different stages of development of the species in order to determine the most effective fungal species.

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