STUDY ON *AMBROSIA ARTEMISIIFOLIA* L. ROOTS COLONIZED BY ARBUSCULAR MYCORRHIZAE IN VARIOUS HERBACEOUS PLANT COMMUNITIES

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Abstract: *Ambrosia artemisiifolia L. is an invasive plant that has spread increasingly in Banat region, expanding their surface very fast, mainly the non-cultivated fields, abandoned pastures, roadsides, along the riversides, near construction sites and even waste areas. Recent researches in the field assumed that arbuscular mycorrhizae (MA) are playing an important role in the spreading and development of this strong allergenic weed. Former researches in this topic have been performed in France on 35 populations of Ambrosia. Therefore, it was noticed by the researchers that the spread of this invasive plant species could be facilitated by arbuscular mycorrhizae. The research aim of the work was to determinate the colonization rate of the Ambrosia artemisiifolia roots with arbuscular mycorrhizae in various herbaceous plant communities from Timisoara area and surroundings. Ambrosia artemisiifolia plants were collected from three locations, respectively the park inside the University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, one abandoned pasture belonging to locality Ghiroda located in the Giarmata Vii village area and a waste area located near the Timisoara International Airport. The mycorrhizal colonization rate of the roots by was determined using special techniques for cleaning and staining of the colonised roots for a good evidencing of the fungal structures. The method applied in this study for the quantification of the colonisation rateof the roots of the analysed root samples of Ambrosia artemisiifolia is onrelativelly common and is using trypan blue for mycorrhizae staining. Quantification of colonization rate of the roots was performed with the method of intersecting grid lines. The colonization rate of the roots of Ambrosia artemisiifolia ranged between 24 and 31%. The highest colonization rate was recorded in the roots of Ambrosia collected from park, respectively 31.02%. The greater colonization rate can be due in this site to the lack of the competition between plant species because it is well known that invasive plants are heavily mycotrophic, while grasses dominant in the park sward are less colonized. The lowest colonization rate was found in the Ambrosia roots collected from the abandoned pasture (24.70%). The fungal structures of the arbuscular mycorrhizae were observed to the microscope. There were observed fungal structures such as hyphae, which were prevalent, arbuscles, vesicles and even spores. Trypan blue staining has provided a good contrast and the mycorrhizal structures were well highlighted at the microscope.*

***Key words*:** *Ambrosia artemisiifolia, arbuscular mycorrhizae, colonization, roots, trypan, arbuscle.*

INTRODUCTION

The importance of invasive species research is given by the fact that their problematic isn't less studied in Romania. The researches in this topic are very complex due to their interdisciplinary characteristic, thus being necessary knowledge in ecology, botany,

phytocoenology *etc.* The researches on the invasive species are the object of some new research directions that have leaded to the appearance of a new branch of the ecology, respectively invasion ecology according with the present problematic of the ecosystems that are suffering deep changes due to the direct and indirect actions of the humans on them. Even if in many cases the changes are almost imperceptible there must to be notice every detail because the implications are most of the times disastrous and the results irreversible.

The agronomic importance of the common ragweed comes from the invasion of the agricultural crops by this weed able to determinate important yield loses. Thus, this species is includes in the quarantine regime, being included in the Official List of the Quarantine Weeds from Romania (ANGHEL *et al*., 1972; IONESCU - ȘISESTI, 1955).

In Europe, as in other regions of the world the number of the invasive species has increased considerably in the last 200 years as a result of the commercial exchanges, tourism *etc.* Thus, in contrast with North America, South Africa, Australia or New Zeeland, the concerns regarding the negative economic and ecological effects of the ecological invasions in Europe started to increase relatively recently (HULME *et al.* 2009).

Ambrosia artemisiifolia is the most important *Ambrosia* species introduced in Europe. This species was registered in almost all the European countries, but with variable densities (DAISIE, 2009). The most seriously affected regions are the centre of Europe (Hungary, Austria and Slovakia), south - east Europe (Romania, Croatia, Serbia) and south Europe (south of France and Italy). In contrast, in the north of Europe *A. artemisiifolia* is rarely found (e.g. Ireland, Scotland, Norway and Sweden), but is expected that the climate changes to facilitate the spreading of this weed in the near future in these regions too (HYVÖNEN *et al*., 2011).

Studies and researches referring to the spread of the species *A. artemisiifolia* L. in our country have been developed in Timis County too by FĂRCĂȘESCU *et* LAUER (2007). They have mentioned the presence of this species in the ruderal and segetal area indicating more many crops where it has been identified, but mainly referring to the presence of the crop in the uncultivated arable land.

Recent researches in field are supposing that the arbuscular mycorrhyzae (MA) plays an important role in the dispersion and development of this strongly allergenic weed. It is well known that in the original habitats its roots are colonised by MA. Researches in this way were performed in France on 35 populations of *Ambrosia*. The colonisation level was comprised between 1 and 40%, depending by the sites from where the plants were collected. Thus, the scientists are supposing that the spreading of this species is facilitated by the MA.

CROWELL and BOERNER (1988), shows that *A. artemisiifolia* is colonized by MA in the United States. In 1991, KOIDE *et* LI have demonstrated that the MA facilitate the absorption of the phosphorus in the *A. artemisiifolia* plant.

In 1993, SCHREINER *et* KOIDE have shown that this plant was able to stimulate the germination of the *Glomus intraradices* spores. The ability to form mycorrhyzae could explain why *A. artemisiifolia* is one of the dominant species from the disturbed habitats from the United States (MEDVE, 1984). FUMANAL *et al*., (2004) highlights the role of the MA in the facilitating the *A. artemisiifolia* invasion.

The main objective of this work is the determining of the *A. artemisiifolia* roots colonization rate. It is well known that the invasive plants are in general strongly mycotrophic, this determinates their capacity to invade new areas in short time.

The approached problem is very actual at worldwide level, there being searched solutions and explanations regarding the factors that are favouring the invasion of *Ambrosia*. One of the research directions in this way is the symbiotic relationship between the MA and common ragweed.

MATERIAL AND METHODS

The colonization rate of the roots with MA was determined in the invasive species *A. artemisiifolia* L. The *A. artemisiifolia* samples were collected from three locations from Timisoara, respectively an abandoned grassland belonging to the locality Ghiroda placed in the perimeter of the village Giarmata Vii, the park from the perimeter of Banat's University of Agriculture Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara and a waste area placed near the Henry Coanda International Airport from Timisoara.

There were collected randomly ten plants of *A. artemisiifolia* from every location from this study.

The root system of the plants was cut in segments of 2.5 cm long. Thus, a root sample was formed from 40 segments, summing 1 m roots. There were analysed 10 root samples of *A. artemisiifolia* for every location.

The MA are growing inside the plant roots needing some technics to make the roots transparent and to highlight the hyphae, arbuscles spores and vesicles to the optic microscope. For the determining of the MA presence and setting of the roots colonization rates the samples were processed, respectively they were washed, cut in segments of 2.5 cm long, hot cleaned with KOH 10% (1 minute) and immersed in HCl 10% (15 minutes), staining with trypan blue solution 0.2%, lactic acid and glycerine (24 hours).

The roots colonization rate was set through the grid lines intersection method (GIOVANETTI *et* MOSSE, 1980), where the roots are dispersed randomly in a Petri plate with the diameter of 9 cm with a grid line. The samples were analysed at stereomicroscope there being quantified the intersection among the roots and gridlines (horizontal and vertical), that appear to being colonized (stained) or being non – colonized (unstained). There was noticed the presence of the hyphae and arbuscles in the cortical tissue.

RESULTS AND DISCUSSIONS

Ambrosia artemisiifolia L. (common ragweed) is an invasive plant highly spread in western Romania, spread mainly on the non-cultivated arable land, abandoned pastures, along the roadsides, along the rivers near to the building areas and the waste areas.

The mycorrhyzal interaction could be a key point in the invasion process of *Ambrosia* through the direct and indirect effects on the concurrence among the plants. The invasive species are recognized now as major factor in the structuring of the plant communities (RICHARDSON *et al*., 2000). The invasion of the exotic plants, as is *A. artemisiifolia* can be a serious threat to the natural ecosystems and not only (PIMENTEL, 2002).

The invasive species are favoured by the vulnerability of the invaded ecosystems by the inter-human exchanges and or by genetic, physiologic and biologic causes (ROY, 1990, PRINZING *et al*., 2002; SAKAI *et al*., 2001). Most of the invasive species are mycotrophic and are adapting successfully in the habitats that are containing MA (RICHARDSON *et al.*, 2000; KLIRONOMOS, 2002; RUDGERS *et al*., 2005).

The purpose of the researches was the setting of the root colonization rate of *A. artemisiifolia* with MA in different habitats from Timisoara and the surroundings.

Quantification of the colonised roots was realised through the method of the intersection of the gridlines (GIOVANETTI *et* MOSSE, 1980). Those authors are recommending that for the analysis of a sample to be used at least 100 intersection of the roots with the gridline. After staining the roots were washed and observed at the stereomicroscope. Every sample was dispersed randomly in a gridded Petri plate of 9 cm diameter.

The AM colonization rate was set by the quantification of the number of the intersections among the gridlines and roots (horizontally and vertically). The intersections considered to be colonized appear coloured and the non-colonized are not coloured (Table 1).

Table 1

AM root colonization rate in *Ambrosia artemisiifolia* L.

No.	Habitat	Average of the AM colonization rate (%)	% I* – infection level	Fungal structures observed at the microscope	Result of the trypan blue staining
1	Wastes area	28,15%	M	Hyphae – predominant Arbuscles Vesicles	Good contrast
2	park	31,02%	M	Hyphae – predominant Arbuscles Vesicles	Good contrast
3	Abandoned pasture	24,70%	M	Hyphae – predominant Arbuscles Vesicles	Good contrast
% I – AM infection level **L (low level), L, <15%** **M (average level), M = 15 – 40%** **H (high level), H, >40%** **N – absent infection**					

Analysing the obtained results we can say that the AM colonization rate of *A. artemisiifolia* roots was situated in the interval comprised between 24 and 31%, the results obtained being in accordance with those obtained by FUMANAL *et al*. (2006), respectively 1 – 40%. The greatest AM colonization rate was registered in the roots collected from park, respectively 31.02% (Table 1). The lowest colonization percentage had the roots collected from an abandoned pasture, respectively 24.7%.

The infection level (I) with AM was average, respectively M = 15 – 40% in the roots collected from those three analysed sites (Table 1).

At the microscope were highlighted fungal structures typical for AM, respectively hyphae that were predominant, arbuscles, vesicles and even spores (figure 1 and 2).

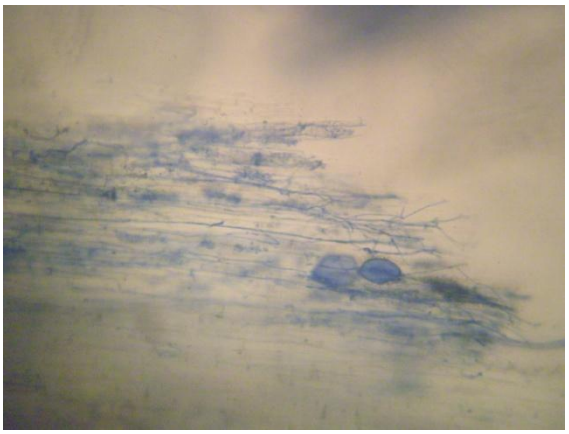

Figure 1. Fungal structures, hyphae and vesicles (photo: OTILIA COTUNA, VERONICA SĂRĂTEANU)

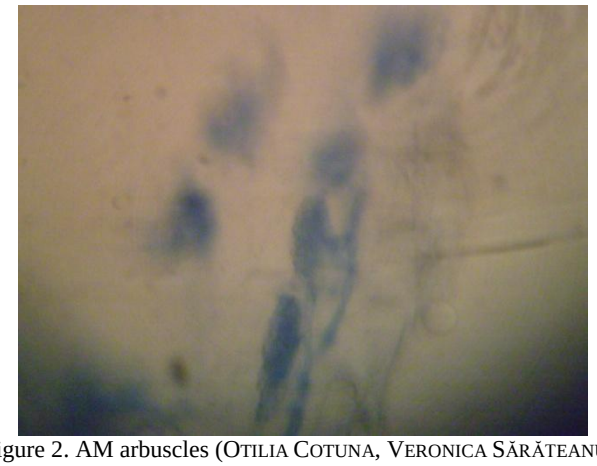

Figure 2. AM arbuscles (OTILIA COTUNA, VERONICA SĂRĂTEANU)

The trypan blue staining has offered a good contrast and the fungal structures have been well highlighted at the microscope.

The obtained results indicates that the symbiotic relationship between the AM fungi and *A. artemisiifolia* is functional and the staining with trypan blue has evidenced well the presence of the arbuscles, even these weren't quantified during this research. The disadvantage of the trypan blue staining is that isn't possible to differentiate the death and live fungal structures.

CONCLUSIONS

The level of infection with AM was moderate in the *Ambrosia artemisiifolia* plants collected from the three vegetation communities (doesn't exceeded 40%). A greater percentage (31.02%) was registered in the roots of *A. artemisiifolia* collected from the park.

The dominant fungal structures were the hyphae and vesicles. The arbuscles were present in all the analysed samples, but in a lower number than the vesicles. The presence of the vesicle and arbuscle structures indicates a viable symbiose between the plants and mycorrhizae. The staining with trypan blue has provided a good contrast of the fungal structures.

The differences regarding the *Ambrosia* roots colonization rate can be due to the concurrence for nutrients with the other species from the vegetation cover of the analysed vegetation communities.

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