SUMMARY OF PHD THESIS

The study of mycorrhizal symbiosis in some grassland ecosystems from the Apuseni Mountains

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SUMMARY

The term mycorrhiza was used, for the first time, by the German scientist B. Frank, specialist in forest species protection, in 1855, who asserts that: “Certain species of trees don’t feed on a regular basis from the soil, but any given portion of their hole root system is in symbiosis with a mushroom spawn, which can play the role of a stepmother and takes the payment for tree’s feeding with food from the soil.” (B. FRANK, 1885, quote by www.biologie.uni-hamburg.de).

Mycorrhiza fungi are symbionts in most of evolved plants roots. This associations vary regarding structure and function, but the most common interactions are those of vesicular-arbuscular type. It is estimated that the percentage of terrestrial plants which form this type of associations exceeds 80% - including many cultivated species (Harley and Harley, 1987).

An important role of this associations is to decrease the input from management system of farms, to optimize nutrition cycles – with a minimum negative ecological impact – assuring increased productions. Therefore this fungi must be looked as a customary component from any sustainable agricultural system.

The proposed project for the PhD thesis is titled: “The study of the mycorrhizal symbiosis in some grassland ecosystems from the Apuseni Mountains”. To achieve the suggested objectives by the project title an experimental field was installed in Ghetari village, Alba county, where it was studied the influence that mycorrhizas had on the plants development and growth, also the effect of some variants of fertilization and treatment on the appearance and development of plant-fungi associations.

Near the existent experiences in mount Apuseni for meeting the proposed objectives during the two year of research, there has been installed another series of experiments in the vegetation house belonging to the University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca, regarding the study of the behavior and strategies of colonization of mycorrhizal fungi in root systems of some cultivated plants in pure culture in controlled environment.
To achieve the research taken by the theme there has been establish the next objectives:

1. the study of the population dynamics of mychorriza vesicular-arbuscular from the researched area(natural meadow subalpine);
2. the fungi specificity towards some botanical species or families;
3. the study of corelation between the factors of climate, tehnology(fertilization, plant treatment, maintenance tehnologies) on evolution of cycle and the capacity of colonization of micorizial fungi;
4. the effect of the features of soil, relief and altitude on the formation on symbiosis micoriziene;
5. the behavior of micoriziene fungi in some sequences of vegetation; the behavior of micoriziene fungi in controlled environment.

**Materials and methods used in experimental field**

To accomplish the objectives, an experimental plot was located on the territory of Ghetari Village, Garda de Sus area, Alba county. The experimental field was divided in 7 variants, 3 repetitions, each variant divided also in 4 other variants.

The experimental factors for the study were:

**Factor A (YEAR) with 2 graduations:**
1. 2010
2. 2011

**Factor B (HARVEST SEQUENCE) with 2 graduations:**
1. 100 days from fertiliyation
2. 150 days from fertilization

**Factorul C (FERTILIZATION) with 7 graduations:**
1. Unfertilized
2. Manure (10t/ha)
3. Manure (10t/ha) + \(N_{50}P_{25}K_{25}\)
4. \(N_{50}P_{25}K_{25}\)
5. Eurofertil mezocalc (120kg/ha) + \(N_{50}\)
6. Eurofertil mezocalc (120kg/ha)
7. Ash (100kg/ha)
Factor D (TREATMENT) with 4 graduations:

1. Untreated
2. Zinc sulfate (1%)
3. Mulch
4. Fungicide (Botran 75 WP, 0.07%)

To facilitate the performance of observation and samples harvesting, the treatment variants were incorporated into fertilization variants.

As biological materials, there were used plants from spontaneous flora existing in the grassland selected to achieve the observations. The selected species, on the floral composition’s study, were highlighted by the highest scale of vegetation cover and prevalent feature in the selected grassland type for the study. So, the grasses were represented by Festuca rubra as a dominant species and the leguminous by Trifolium repens.

The fertilization and the treatments were made every year in the midDL/LSDe of April, after the snow melting and before the starting of vegetation growth of the plants from the experimental plot (Fig. 5.2.).

During the period of the year, the protocol of harvesting the samples was established, separated on two sequences of vegetation. The first sequence of vegetation coincides with the moment of mowing the plot, 100 days from the fertilization and treatments, and the second sequence, establish to 150 days and which was considerate the moment of the beginning of the decline in growth and development of the plants, which means preparation for winter.

Materials and used methods in controlled environment

During the year of 2011, a series of experiments were located in the controlled environment from the vegetation house belonging to the University of Agricultural Sciences and Veterinary Medicine from Cluj, in order to study the parameters of mycorrhizal colonization differentiated on sequences of vegetation, also to find some
points of reference which can relate to the field’s data, to form a complete image on functioning of the mycorrhizal fungi.

The experiments lasted 150 days, the used plants being the same as in the experimental field, *Trifolium repens* and *Festuca rubra*.

For the first experiment initiated the harvesting of the variants was made progressive, during a period of 10 days from one to each other, starting with the first harvesting, scheduled 10 days after plants emergence.

The 15 variants were located in 3 repetitions and the seeding was made on a *terra rosa* soil, identical with the experimental field.

A second experiment compound from 7 variants was located to analyze the fertilizations effect upon the parameters of colonization in controlled environment from vegetation house.

The fertilization was made by using the same experimental protocol like in the case on the field experiments, the soil being identical to the one in the experimental field from Ghețari village.

The harvesting of the root samples was made in two sequence, at 100 and 150 days from the plants emergence.

**Materials and methods used in laboratory**

For the microscopically analysis of the vesicular-arbuscular fungi there were used two methods, based on the method developed by Vierheilig et al. (1998), to which have been broth a series of changes and the results indicated a better efficiencies of the color (Stoian and Florian, 2009).

Estimation of mycorrhizal colonization was made according to parameters proposed by Trouvelot ey al. (quote by www2.dijon.inra.fr), notes granted to colonization being according to intraradicular micelium development and arbuscules abundance.

**The analysis of colonization parameters in pure culture of *Festuca rubra* on a vegetation period of 150 days**
The interval of 40 – 70 days from plant emergence is marked by an increase of the colonization intensity at 40 days until 60.61%, followed by a decline to about 32.39% and again an increase until 69.50% to 70 days. In the same way arbuscules abundance fluctuate in root system, the percentage being 26.75% at the beginning of the interval, followed by a decline to 0% (at 50 days), and then an increase until 51.79% at the end of the interval. During this period the value of colonization frequency remains fixed at 100%. The sequence of 80 days highlights by a new decline of frequency of colonization, a decline of intensity until 3.20% and the absence of arbuscules.

The increased value of arbuscules abundance indicates a bigger intensity of exchange processes of nutrients at the interface between root’s system cells of plants and the colonizing fungus hiphae, a period of increased activity in development and growth of plants.

**The analysis of colonization’s parameters in pure culture of *Festuca rubra***

**Differentiated fertilized on a vegetation period of 150 days**

From the data’s correlation, regarding the period of harvesting and fertilization on frequency of root system colonization of plants of *Festuca rubra*, a negative influence of fertilization can be noticed in the first period of harvesting, comparative with the second harvesting where the fertilization variants with manure and ash influenced in a positive way the frequency of colonization.

From the study of the first harvesting period, a significantly negative decrease of colonization intensity regarding the fertilized variant with Eurofertil mezocalc (120kg/ha) and N50, the other variants recording significantly negative differences toward control variant.

In the second period of harvesting, the fertilization influenced in a negative way the intensity of colonization to variants fertilized with manure and NPK and NPK, the difference toward control variant being very significantly negative.

The variants 2, 5, 6 and 7 had differences very significantly positive, which indicates a beneficial effect of fertilization on intensity of colonization.
Correlating the data from the two period of harvesting we can find that the fertilization with manure + NPK and NPK (variants 3 and 4), has a negative effect on colonization throughout the hole interval of growth in the study, with significantly negative differences from the variant not fertilized. Regarding the other variants, the fertilization has a positive effect on the degree of colonization starting with the second period of harvesting.

The analysis of colonization parameters in pure culture of Trifolium repens on a vegetation period of 150 days

Regarding the appearance of frequency of colonization, at 10 days from the emergence mycorrhizal fungus succeeds to colonize approximately half of the root system (52.22%), but with an intensity of 2.07% and an arbuscules abundance of 0.11%. The colonization parameters increases beginning with the sequence of vegetations of 20 days , the frequency getting to the value of 100%, which is kept until 70 days from the heave.

Over the same interval the intensity of colonization reaches the value of 76.17% at 20 daysand 71% at 30 days, decreasing after that to a value of 42.28% at 70 days from the plants emergence, meanwhile the arbuscules abundance reaches a value of 28.60% at 20 days, respectively 35.84% at 30 days, but decrease to 0.13% during the 50 days sequence and grow again until a value of 14.66% at 70 days.

The moment of establishment of colonization in the radicular system is revealed by small values of frequency and intensity of colonization, respectively of arbuscules abundance. At the same moment with the growth and development of plants, the request for nutrients causes a continous growth of the radicular system.

The analysis of colonization’s parameters in pure culture of Trifolium repens differentiated fertilized on a vegetation period of 150 days

During the vegetation sequence of 100 days it is noticeable a decreasing of frequency of colonization values at fertilization variants 2, 3 and 7, the differences being very significantly negative from unfertilized variant.
From analysis of the results obtained at the second vegetation sequence it is observed an increase of colonization frequency at all the fertilizing variants.

Fertilizing with manure, manure combined with chemical fertilizers, respectively with ash, had a negative influence over intensity of mycorrhizal colonization during the first studied sequence.

On the other part, the variant fertilized with NPK and the variant fertilized with eurofertil mezocalc had a good impact over colonization intensity, the registered differences being assured as very significant positive.

Fertilization had a negative influence over mycorrhizal colonization degree in case of variants 3 and 7, the differences being significantly negative at variant 3, respectively distinct significant negative at variant 7 from unfertilized variant.

The comparative analysis of colonization parameters in pure culture of Festuca rubra and Trifolium repens on a vegetation period of 150 days

Comparing the registered values, it can be observed that in case of species Festuca rubra the interval 20 – 70 days shows a slightly increase of the colonization frequency value, meanwhile to the species Trifolium repens the increase from the control sequence in the same interval is assured to be very significantly positive to all sequences.

The interval 80 – 90 and 120 – 140 days from the emergence indicate a decrease of colonization frequency from control sequency in case of Festuca rubra species, comparative to Trifolium repens where the frequency grow.

Over all the studied vegetation period of 150 days, the colonization intensity on Trifolium repens species had bigger values than the control sequence, except the interval 130 – 150 days, where the intensity decrease but with non-significantly differences.

By comparison, in case of Festuca rubra species, the intensity has fluctuating values, the statistical differences alternate between very significantly positive and significantly negative from control, and in case of 100 – 150 days interval the values presents very significantly negative differences to control sequence.
The comparative analyses of the parameters of colonization in pure culture of *Festuca rubra* and *Trifolium repens* fertilized differentiated on a period of vegetation of 150 days

Regarding the first sequence of vegetation, the values of frequency appearance of root’s system colonization to the species *Festuca rubra* to each variant it’s shows differences very significantly negative from the control variant, meanwhile to the species *Trifolium repens*, just the variants 2, 3 and 7 had values very significantly negative, the other variants having insignificant values.

Regarding the intensity of the observed colonization to the two species, the table 6.26. presents a synthetic analyses of the registered values during the period of vegetation of 150 days from the emergence divided in two sequences of vegetation (100 and 150 days).

The observation’s data made in the first sequence of vegetation indicates a negative influence of fertilization upon both species to the variants of fertilizations 2, 3 and 7, the differences from the control being very significantly negative. From the data’s analyses made at the variants of fertilization 4 and 6, it is noticeable a different influence of fertilization upon the two species. While to the *Festuca rubra* the fertilization decreases the intensity of colonization, the differences being very significantly negative, to *Trifolium repens* it is noticeable a beneficial influence, obtaining increase values then the one observed at the unfertilized variants, the differences being very significantly positive.

Regarding the species *Festuca rubra* the registered data to the sequence of 100 days indicates a negative influence of fertilization upon the degree of colonization to all the variants, the differences being very significantly negative, except variant 5 to which it has been observed a difference distinct significantly negative from the unfertilized variant. The negative influence of fertilization is found also to the species *Trifolium repens*, but only to the variants 2, 3 and 6.
The analysis of colonization parameters at species *Festuca rubra* in the years 2010-2011

The comparison between values of colonization frequency recorded in the two years of study shows a positive influence of fertilization upon frequency of colonization at variant 5, fertilized with Eurofertil mezocalc and nitrogen and this variant is the only one that record very significantly positive differences in the both years.

On the other side is situated the fertilization with manure (10t/ha) on variant 2 which influence in a negative way the frequency of mycorrhizal colonization, in year 2010 the value of difference being very significantly negative compared to control; in the year 2011 the situation is a little bit better, the difference being distinct significantly negative.

Regarding the influence of treatment over frequency of mycorrhizal colonization on the both years of study, the values from table 7.20 indicate a positive influence of treatment with Botran 75 WP, the recorded values being very significantly positive from untreated variant.

The mulch combined with fertilization had a benefic effect over the colonization degree, in year 2010 the variants 2, 3 and 5 recording values bigger than value observed at control variant and differences were assured as very significantly positive at variant 3, distinct significantly positive at variant 5 and significantly positive at variant 2. Year 2011 indicates a weak influence of mulch and fertilization and none of the variants had significantly differences to control variant.

The analysis of colonization parameters at species *Trifolium repens* during the years 2010-2011

The comparison between the values of frequency from the two years of study shows a beneficial influence of fertilization to variant 5 fertilized with NPK, being the only variant that shows the differences very significantly positive in both years.

To all the untreated variant but fertilized it is noticeable a value near the value of control variant, while the variants 2 and 5 present a distinct difference significantly negative at variant 2 and a significant negative difference at variant 5.
In the year 2011 with the exception of variant 4 all the variants had differences very significantly negative from control variant.

Regarding the influence of zinc sulfate applied at the same time with the fertilization, in the year 2010 stands differences very significantly positive of the registered values at the variants 3,4 and 7. In the year 2011, it’s noticeable a continuance of the negative influence upon the colonization frequency to the variants 4 and 7, showing again very significantly positive differences, meanwhile the rest of the variants recorded differences very significantly negative (variants 2,3 and 6) and the variant 5 a difference distinct significantly negative.

The mulch with the fertilization had an inhibitory effect upon the colonization frequency, in the year 2010 only the variant 4 didn’t register a significantly difference, the values noticed at the other variants are statistically assured to be significantly negative.

The year 2011 brings a slightly modification to the level of frequency of the colonization, variants 4 and 7 having a difference very significantly positive from the control, meanwhile the other variants have differences very significantly negative from the control variant.

Regarding the influence of zinc sulfate applied the same time with the fertilization upon the degree of colonization, in the year 2010 it’s noticeable differences distinct significantly positive to the values 4,6 and 7, and distinct significantly positive to the variant 3, the other variants didn’t had significantly differences. In the year 2011, it’s noticeable a negative influence upon the degree of colonization to the variants 2,3,5 and 6 statistical assured to be distinct significantly negative, the other variants having differences very significantly positive from the untreated and unfertilized variant.

The variant 3 has a difference very significantly positive from the control variant. The year 2011 indicates a beneficial influence regarding variants 3 and 4, the differences are very significantly positive, but regarding the other variants it’s noticeable decreases value then the value of the control variant, the variants 2 and 6 are assured again with differences very significantly negative.
In the year 2010 all fertilized and treated variants with fungicide had differences very significantly negative from the control, except the variant 2 in which the difference was significantly negative, while in the year 2011 it’s noticeable the differences very significantly negative to almost all the variants, except variant 3 which didn’t had significantly differences.

Conclusions

Clustered analysis of mycorrhizal fungi colonization strategy in the radicular system of Festuca rubra, based on the values of frequency, intensity and arbuscules abundance, indicating the existence of two stages of colonization. A stage marked by a process of storage of nutrients, production of vesicles and superficial colonization and a second stage characterized by processes of nutrient transfer and intraradicular hyphal extension.

Comparison of the values recorded on colonization degree at species Trifolium repens in a growing season of 150 days, in a controlled environment, in terms of fertilization indicate the most favorable influence of fertilization as the variant with eurofertil mezocalc while the variant fertilized with ash was highlighted as the worst.

The complex of factors represented by years of experimentation, treatments and fertilization seen in terms of influence on the degree of colonization of the root system of plants from Festuca rubra revealed the most favorable influence at the combination of climatic conditions in 2010 x treatment with fungicide x absence of fertilization and the worst influence found in combination conditions of year 2011 x treatment with zinc sulphate x fertilization with manure.

The complex of factors represented by years of experimentation, treatments and fertilization seen in terms of influence on the degree of colonization of the root system of Trifolium repens plants revealed the most favorable influence at the combination of climatic conditions of 2010 x treatment with zinc sulphate x fertilization with ash and the worst influence found in combination conditions of year 2011 x treatment with fungicide x fertilization with eurofertil mezocalc.