IMPACT OF FERTILIZER AND TREATMENT RECIPE ON MYCORRHIZAL FUNGI PROGRESS IN THE ROOT SYSTEM OF *Festuca rubra* PLANTS

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Abstract

Mature ecosystems are characterized by a simultaneous evolution of plants and mycorrhizal type radicular symbionts, extraradicular hyphae produced by mycorrhizal fungi connecting higher plants through interradicular networks and thus balancing the transfer of nutrients across the ecosystem. Biodiversity of ecosystems is based on the balance created by mycorrhizal networks. Fertilization can cause a destabilization of the ecosystem, favoring the preferential allocation of nutritional resources to dominant plants in grass cover. Integrating fungicides in treatment recipe act to preserve nutritional balance, and zinc for increasing the amount of water absorption. The action of treatments superposed on fertilization recipes is enhanced by environmental conditions. The application of chemical fertilization with a nitrogen base reduces the intensity of the colonization, and superposing a treatment with fungicide stabilizes the frequency with which the *Festuca rubra* roots are colonized to less than 70%. Effect of fertilization and treatment recipes is amplified by environmental factors. Mycorrhizal colonization is enhanced by fertilization with manure and fungicide treatments, while zinc sulfate overlapped on the effect of manure acts to reduce colonization. Fungal symbionts response to experimental factors falls within the limits of four clusters. Overlapping chemical fertilization with nitrogen over fungicide treatment leads to reduced values of the colonization parameters in the second year of experimentation. A beneficial effect for the installation of symbionts in the root system of *Festuca rubra* plants is played by the chemical fertilizing, in the first experimental year conditions, over which is overlapped a treatment with zinc sulfate.

Key words: fertilization, mycorrhizal colonization, zinc sulphate, fungicide

In matured ecosystems, the mycorrhizal activity adjusts the composition and functionality of vegetal communities, by effectively allocating food sources through a hyphal network, interradicular developed, in which the mycorrhizal fungi are radicular symbiotic of the superior plants. The major contribution of vesicular – capered fungus is placed into the absorption and transfer niche of the nutritive elements in the soil. The fungi are developing here an extra-radicular hyphal network capable of exploring soli sections to which plants do not have direct access.

The growth and development of plants, the tolerance to dryness induced stress or even the endurance enhance to disease and harmful insects, is owed to the presence in the radicular system of the vesicular – capered fungus. The temporally evolution of superior plants took place concomitantly with the one of mycorrhizal fungi. Currently, this represents an important percentage from the soil microbial biomass (Jayne and Quigley, 2013, Saia *et al.*, 2014, Mariotte *et al.*, 2013).

Tolerability to dryness of superior plants is in tight connection with the efficiency to access and utilize the soil's water resources, process allowed by the zinc element. Applying this microelement may have positive consequences on the development of radicular colonisation level, and hence expanding the water absorption surface (Cavagnaro *et al.*, 2010, Watts-Williams et al., 2014).

At an ecosystem level, the plant's incapacity to reach difficult accessible resources, as well as the effective transfer towards symbiotic plants, allows mycorrhizal fungi to bring down the inputs in the management system, optimizing the nutrition cycles and increasing the production.

Still, in time, the capacity to adjust nutrition resources at an ecosystem level, may lead to an imbalance between the plants communities, in the favour of those species with mycorrhizal association capability. After a certain level, the biodiversity may be irrecoverably affected, some species threatened with extinction. In this scenario, the usage of fungicides may have a positive effect, by reducing the mycorrhizal colonization level, maintaining thus the ecosystem's equilibrium

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(Ehrmann and Ritz, 2013, Varma, 2008).

MATERIAL ŞI METHODS

The information related to mycorrhizal colonization progress was collected from an experimental field, with 6 degrees of fertilization. Along with the unfertilized witness (F1), 5 fertilization levels were used: fertilization with manure (F2) – 10 t/ha; nitrogen supplemented manure (F3) – 10 t/ha + NPK = 50:25:25, mineral with NPK (F4) - 50:25:25; mineral with Eurofertil mezocalc (F5) – 120 kg/ha + N = 50; mineral with Eurofertil mezocalc (F6) – 120 kg/ha.

Each fertilization recipe was supplemented with a zinc sulphate base treatment (T1 – 1.0%), and a fungicide (T2 – Botran 75 Wp – 0.07%). The objective was to assess the variation produced on the Frequency (*freq%*) and Intensity *(int%)* levels of the mycorrhizal colonization in the radicular system of *Festuca rubra*.

The analysis of evolution of colonization parameters in fertilization and treatment conditions uses data obtained in 2010 (A1) and 2011 (A2), the ecological conditions being calculated for the maximum sequence of the vegetation period.

The experimental concept, the experimental field design and the data collection methodology were presented by Stoian (2011). The statistical analysis level additions are underlining the individual effect of experimental factors over the years of trail (Vidican *et al.*, 2013).

Evaluating the differences between colonization levels for the experimental variants was performed with ad-hoc – Fisher LSD (Statsoft, 2012) comparative analysis. The assessment of the fertilization level, treatment and ecological factors over the colonization parameters was performed with a cluster type analysis. For assessing the data and projecting the graphs, the R Statistics (R Core Team, 2013) soft – "vegan" package (Oksanen et al., 2013) was used.

RESULTS AND DISCUSSIONS

The combined effect of fertilization and treatment applied during 2010, has produced significant fluctuations of the mycorrhizal fungi progress in the roots of *Festuca rubra* plants (Table 1; Table 2). The colonization intensity is strongly decreasing with the integration of chemical fertilizers in the formula (Table 1), the highest values being recorded for manure fertilization (V2). High values of the colonization intensity were recorded for the unfertilized variant to both treatments (V1), with highly significant differences in most cases.

The lowest level of intensity was recorded to the nitrogen supplemented variants (V3 and V5) combined with a zinc sulphate treatment (*table 1*). Up against these variants, all fertilization and treatment combinations have registered significant differences.

Table 1

Fertilization and treatment effects over the colonization intensity (2010)

F			1	1	2	2	3	3	4	4	5	5	6	6
	T		1	2	1	2	1	2	1	2	1	2	1	2
		Int	20.17	26.93	14.18	31.31	5.24	15.07	11.18	9.20	5.50	6.09	17.08	6.76
1	1	20.17		p<0.001	0.001	p<0.001	p<0.001	0.003	p<0.001	p<0.001	p<0.001	p<0.001	0.061	p<0.001
1	2	26.93			p<0.001	0.010	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
2	1	14.18				p<0.001	p<0.001	0.578	0.068	0.004	p<0.001	p<0.001	0.078	p<0.001
2	2	31.31					p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
3	1	5.24						p<0.001	0.001	0.019	0.870	0.595	p<0.001	0.345
3	2	15.07							0.021	0.001	p<0.001	p<0.001	0.213	p<0.001
4	1	11.18								0.221	0.001	0.004	0.001	0.010
4	2	9.20									0.027	0.060	p<0.001	0.133
5	1	5.50										0.712	p<0.001	0.433
5	2	6.09											p<0.001	0.675
6	1	17.08												p<0.001
6	2	6.76												

p < 0.05 * ° ***p < 0.01*** ** °° ***p < 0.001*** *** °°°

Applying a fungicide treatment to the manure fertilized variants (V2 and V3) has acted towards increasing the progress intensity of the radicular colonization. The recorded values were 2 times higher in the case of manure fertilized variant (V2) and 3 times higher in the case of nitrogen supplemented manure fertilization (V3) besides the correspondent fertilized variants which

were treated with zinc sulphate (*table 1*), differences being statistically proved as highly significant.

The nitrogen addition to the manure fertilization (V3) triggers a considerable decline of the colonization intensity level when compared with the unilateral fertilized variant with manure (V2), for both treatment cases (Table 1). The same phenomenon does not repeat for the chemical fertilizations (V4, V5 and V6), where applying treatments has a reduced effect over the intensity, and the differences between the 2 treatments on the same variant are much reduced.

Treatments applied during 2010 do not reduce the colonization frequency for unfertilized variants, the recorded values in the plant's roots being 100% (*table 2*). At manure fertilized variants (V2 and V3), the zinc sulphate treatments has led to a decrease in colonization frequency, in comparison with the fertilized variants when treated with fungicide.

Table 2

Fertilization and treatment effects over the colonization frequency (2010)

F			1	1	2	2	3	3	4	4	5	5	6	6
	T		1	2	1	2	1	2	1	2	1	2	1	2
		Freq	100.00	100.00	93.33	100.00	73.33	100.00	96.67	67.78	85.56	65.56	86.67	93.33
1	1	100.00		1.000	0.057	1.000	p<0.001	1.000	0.327	p<0.001	p<0.001	p<0.001	0.001	0.057
1	2	100.00			0.057	1.000	p<0.001	1.000	0.327	p<0.001	p<0.001	p<0.001	0.001	0.057
2	1	93.33				0.057	p<0.001	0.057	0.327	p<0.001	0.028	p<0.001	0.057	1.000
2	2	100.00					p<0.001	1.000	0.327	p<0.001	p<0.001	p<0.001	0.001	0.057
3	1	73.33						p<0.001	p<0.001	0.109	0.001	0.028	0.001	p<0.001
3	2	100.00							0.327	p<0.001	p<0.001	p<0.001	0.001	0.057
4	1	96.67								p<0.001	0.003	p<0.001	0.006	0.327
4	2	67.78									p<0.001	0.512	p<0.001	p<0.001
5	1	85.56										p<0.001	0.742	0.028
5	2	65.56											p<0.001	p<0.001
6	1	86.67												0.057
6	2	93.33												

p < 0.05 * ° ***p < 0.01*** ** °° ***p < 0.001*** *** °°°

On variants where nitrogen based chemical fertilisation was applied (V4 and V5), the fungicide treatment acted towards reducing the colonization frequency up to a value of 65%, while applying the zinc sulphate to the same variants maintained the frequency values over 85% (Table 2). Compared to the variants fertilized with chemical nitrogen (V4 and V5), the manure fertilized variants have registered significant differences.

The ecological conditions of year 2010 have intensified the fertilization and treatment effects over the mycorrhizal colonization parameters, the differences between variants being statistically ensured (*table 3* and *table 4*). The zinc sulphate treatment applied to the unfertilized variant has reduced the intensity of the mycorrhizal colonization up to 1.77% (V1), while applying fungicide to the same variant maintained the intensity level to 10.90% (*table 3*).

The highest value recorded for the intensity level, was identified at the manure fertilized variant (V2) and treated with fungicide, the 27.20% value of this parameter being significantly higher than all the other experimental variants (Table 3). Adding nitrogen to the manure fertilization (V3) in the conditions of a fungicide treatment has stabilized the intensity of colonization to a 15.11% value, almost 3 times higher than the value recorded for the same variant treated with zinc sulphate.

The nitrogen chemically fertilized variants (V4 and V5), while undergoing the fungicide treatment, have recorded values of intensity under 1%, when compared with applying the zinc sulphate treatment, case in which the values exceed 8% (*table 3*). This phenomenon is not repeted to chemically fertilized variant with no nitrogen (V6), applying the treatments to it having a much reduced influence.

The colonization frequency in radicular system of the Festuca *rubra* plants in 2011 reached values over 90% only for the manure fertilized variants (V2 and V3). Additional to the

fertilization, a fungicide treatment was applied (*table 4*). At the same fertilization variants, the zinc sulphate treatment acted towards the frequency's decrease to a level of 64.45% (V3) and 48.89% (V2), the effect being more significant in the manure unilateral fertilization (V2) case.

Table 3

Fertilization and treatment effects over the colonization intensity (2011)

F			1	1	2	2	3	3	4	4	5	5	6	6
	T		1	2	1	2	1	2	1	2	1	2	1	2
		Int	1.77	10.90	1.24	27.20	4.84	15.11	10.16	0.89	8.08	0.85	4.99	6.50
1	1	1.77		p<0.001	0.412	p<0.001	p<0.001	p<0.001	p<0.001	0.174	p<0.001	0.155	p<0.001	p<0.001
1	2	10.90			p<0.001	p<0.001	p<0.001	p<0.001	0.251	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
2	1	1.24				p<0.001	p<0.001	p<0.001	p<0.001	0.578	p<0.001	0.532	p<0.001	p<0.001
2	2	27.20					p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
3	1	4.84						p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	0.821	0.014
3	2	15.11							p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
4	1	10.16								p<0.001	0.003	p<0.001	p<0.001	p<0.001
4	2	0.89									p<0.001	0.945	p<0.001	p<0.001
5	1	8.08										p<0.001	p<0.001	0.019
5	2	0.85											p<0.001	p<0.001
6	1	4.99												0.024
6	2	6.50												

p < 0.05 * ° ***p < 0.01*** ** °° ***p < 0.001*** *** °°°

Table 4

Fertilization and treatment effects over the colonization frequency (2011)

F			1	1	2	2	3	3	4	4	5	5	6	6
	T		1	2	1	2	1	2	1	2	1	2	1	2
		Freq	68.89	65.56	48.89	98.89	64.45	96.67	72.22	44.44	82.22	51.11	64.45	58.89
1	1	68.89		0.279	p<0.001	p<0.001	0.153	p<0.001	0.279	p<0.001	p<0.001	p<0.001	0.153	0.003
1	2	65.56			p<0.001	p<0.001	0.715	p<0.001	0.037	p<0.001	p<0.001	p<0.001	0.715	0.036
2	1	48.89				p<0.001	p<0.001	p<0.001	p<0.001	0.153	p<0.001	0.467	p<0.001	0.003
2	2	98.89					p<0.001	0.467	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
3	1	64.45						p<0.001	0.016	p<0.001	p<0.001	p<0.001	1.000	0.077
3	2	96.67							p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
4	1	72.22								p<0.001	0.003	p<0.001	0.016	p<0.001
4	2	44.44									p<0.001	0.036	p<0.001	p<0.001
5	1	82.22										p<0.001	p<0.001	p<0.001
5	2	51.11											p<0.001	0.016
6	1	64.45												0.077
6	2	58.89												

p < 0.05 * ° ***p < 0.01*** ** °° ***p < 0.001*** *** °°°

The zinc sulphate and fungicide treatments applied to unfertilized variants (V1), established a colonization frequency level values between 60 – 70%. Similar values were recorded only in the case of nitrogen supplemented manure fertilized variant (V5) and Eurofertil mezocalc fertilization (V6), in zinc sulphate treatment conditions (*table 4*).

Differences between the treatments applied to NPK fertilized variant (V4) and Eurofertil mezocalc nitrogen supplemented fertilization (V5) were almost 30%, applying the fungicide acting towards reducing the colonization frequency in the radicular system of *Festuca rubra* specie (*table 4*).

The cluster analysis of the recorded experimental data has indicated a good segmentation of the fertilized variants subject to fungicide or zinc sulphate treatments, on the basis

of frequency and intensity values of the radicular system colonization (*figure 1*).

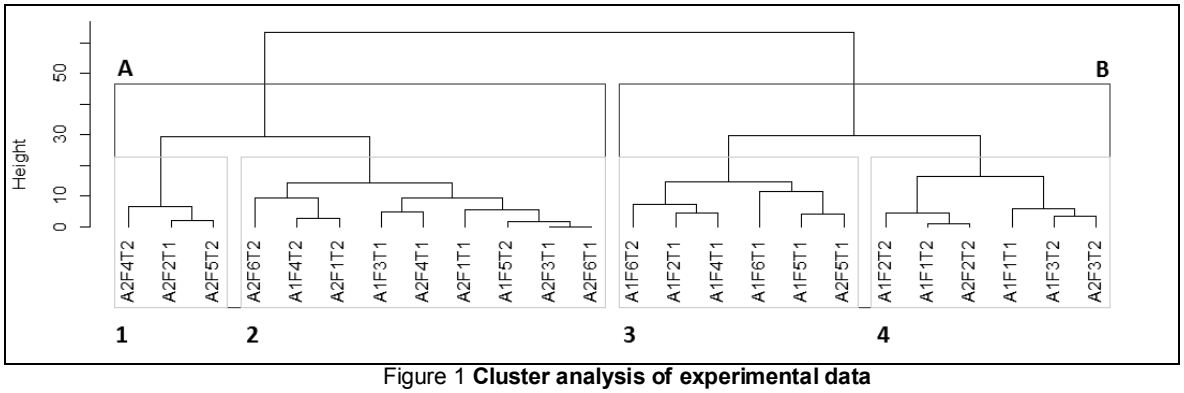

Figure 1 **Cluster analysis of experimental data**

At cluster A level were identified the 2010 chemical fertilized variants (A1) with NPK (F4) and Eurofertil mezocalc supplemented with nitrogen (F5) combined with a fungicide based treatment, and the manure supplemented with nitrogen variant (F3) when treated with zinc sulphate (*figure 1*).

The defining values of the colonization frequency for cluster A are recorded under 75%, and the intensity of colonization in the radicular system under 10% in most cases. The exceptions are the unfertilized fungicide variant (A2F1T1), and the NPK fertilized variant, when treated with zinc sulphate (A2F4T1). Both cases have recorded intensity values of over 10% during the 2011 experiment (*figure 1*; *table 1*).

B cluster is characterized by high values of the colonization's frequency, over 90%, with the exception of variants fertilized based on Eurofertil mezocalc combined with zinc sulphate treatment. These variants recorded a colonization frequency of 80 – 90% (*figure 1*; *table 5*). In this cluster, the maximum intensity value is recorded – 31.31%, to the 2010 manure fertilized and fungicide treated variant (A1F2T2).

Table 5

Values of mycorrhizal colonization parameters during experimental years based on the applied fertilization and treatment

Fert	Trat	Year	plot	int	freq	Year	plot	int	freq
1	sulph	2010	A1F1T1	20.17	100.00	2011	A2F1T1	1.77	68.89
1	fung		A1F1T2	26.93	100.00		A2F1T2	10.90	65.56
2	sulph		A1F2T1	14.18	93.33		A2F2T1	1.24	48.89
2	fung		A1F2T2	31.31	100.00		A2F2T2	27.20	98.89
3	sulph		A1F3T1	5.24	73.33		A2F3T1	4.84	64.45
3	fung		A1F3T2	15.07	100.00		A2F3T2	15.11	96.67
4	sulph		A1F4T1	11.18	96.67		A2F4T1	10.16	72.22
4	fung		A1F4T2	9.20	67.78		A2F4T2	0.89	44.44
5	sulph		A1F5T1	5.50	85.56		A2F5T1	8.08	82.22
5	fung		A1F5T2	6.09	65.56		A2F5T2	0.85	51.11
6	sulph		A1F6T1	17.08	86.67		A2F6T1	4.99	64.45
6	fung		A1F6T2	6.76	93.33		A2F6T2	6.50	58.89

The high fluctuation of colonization level inside each cluster has requested a second segmentation, much stronger, of the 2 clusters, resulting 4 clusters (1; 2; 3; 4), much more balanced from a comparison range point of view (*figure 1*).

Cluster 1 includes 3 experimental variants, defined by very low colonization frequency and intensity levels (*figure 1*; *table 5*). In ecological conditions of 2011, the NPK fertilization (A2F4) and Eurofertil mezocalc supplemented with nitrogen fertilization (A2F5), added to a fungicide treatment (T2), the frequency values was 44.44%, and 51.11%; and the intensity level was under 1%. The zinc sulphate treatment combined with an organic fertilization (A2F2T1), in the same ecological conditions, converts to reduced colonization intensity (1.24%) and a frequency of below 50%.

Inside cluster 2, the colonization frequency ranges between 58.89% - 73.33%, while the intensity records values between 1.77% - 10.90%, indicating a much stronger colonization than the one observed in cluster 1, but shallow in

comparison with the development of the complete radicular system of mycorrhizal fungi (*figure 1*, *table 5*).

The colonization intensity follows closely the frequency's values, cluster 3 being recognized by intensity values of 5.50%, and a maxim of 17.08% at the variant with a zinc sulphate based treatment and fertilized with Eurofertil mezocalc (A1F6T1), in the 2010 ecological conditions (Fig 1, Table 5). The maximum frequency level was recorded for the NPK fertilized variant, treated with zinc sulphate (A1F4T1). Only one variant from 2011 was included in this cluster, with a frequency value of 82.22% and 8.08% intensity, based on the fertilization effect of the Eurofertil mezocalc supplemented with nitrogen, combined with a zinc sulphate based treatment.

Cluster 4 includes the highest colonization frequency variants, 100%, for all unfertilized variants in 2010; and over 95% to fungicide treated and fertilized with manure (A2F2T2) and manure supplemented with NPK (A2F3T2), under the climatic conditions of 2011 (*figure 1*, *table 5*).

CONCLUSIONS

By applying fungicide over fertilization with manure, even after a nitrogen supplementation, maintains a high level of mycorrhizal colonization. A zinc sulphate based treatment decreases the colonization parameters when subjected over the same fertilization network.

The nitrogen based chemical fertilization, when completed with a zinc sulphate treatment; produced a positive effect on the intraradicular progress of the mycorrhizal colonization. The values are much higher than in the case of applying a fungicide treatment to the same fertilization variant.

The ecological factors are significantly affecting the stage of mycorrhizal colonization in comparison with the technological factors, which produces the split into clusters, framed by the cumulated values of colonization parameters. The technological factors are acting towards the raise of the decline of the colonization potential.

By introducing a fertilization and treatment complex, adapted to the climatic conditions, with the purpose of reducing the individual influence of only one factor and acting as a sum of all factors, may coordinate the mycorrhizal fungi colonization towards maximum values of frequency and intensity. This phenomenon was highlighted in the described clusters.

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