Original Research

Impact of Chosen Bare Root Nursery Practices in Scots Pine Seedling Quality and Soil Mites (Acari)

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Abstract

The purpose of our study was to determine the effect of microirrigation and organic fertilization on the vitality of Scots pine (*Pinus sylvestris* L.) seedlings and their mycorrhizal structure, as well as the occurrence of soil mites (Acari) after treatment of edaphon inoculation. Nursery experiments were carried out in 2003-05. It was found that studied factors influenced seedling vigour, mycorrhizal colonization, soil properties and the occurrence of mites.

Keywords: microirrigation, organic fertilization, Scots pine seedling vitality, mycorrhiza, occurrence of mites

Introduction

The establishment and performance of outplanted tree seedlings may be significantly affected by ectomycorrhizal fungi, which are key players in biogeochemical cycles and contribute to host plant nutrition [1-4]. Land formerly used for agricultural purposes may lack ectomycorrhizal inoculum due to the absence of suitable host trees, thus if an afforestation is to be beneficial, outplanted seedlings should have appropriate mycorrhiza on roots.

It has been observed in many Polish bare-root nurseries that the number and composition of mycorrhiza has decreased [5]. Disappearance of ectomycorrhizal fungi is connected with excessive soil alkalization, fertilization, mechanical soil tillage, and pesticide application. These factors can lead to microbiological changes of forest soils. Hence, an improvement of nursery soil by inoculation with edaphon derived from forest soil seems to be a good solution.

Irrigation is one of the most important cultural practices in forest nurseries. The main objective of nursery irrigation is to avoid unwanted seedling moisture stress and its negative consequences for seedlings [6]. The use of irrigation enables a systematic supply of water to young plants, which also ensures adequate moisture for edaphon. This is very important for humification and mineralization as well as for nutrient uptake. Irrigation is conducted mostly with the use of sprinklers. In recent years, water-saving irrigation systems have been tested to improve water management in forest nurseries [7].

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The organic matter component of soils is a decisive factor when evaluating their suitability for seedling production in forest nurseries [8]. High soil productivity in forest nurseries is determined by supply of organic fertilizers, such as compost. In this trial, compost produced from sewage sludge was used. The natural use of sewage sludge is justified from an ecological point of view [9].

The cap humus of forest soils is inhabited by an abundant number of micro-artropods. One of the most abundant groups of mesofauna are mites which play a number of important roles in forest ecosystems. Most of them, as saprophages, take roles in decomposition of organic matter, which influences the growth of trees.

The aim of the study was to determine the effect of micro-irrigation (micro-jet sprinkling and drip irrigation) and organic fertilization on the vitality of Scots pine (*Pinus sylvestris* L.) seedlings and their mycorrhizal structure as well as the occurrence of soil mites (Acari) after edaphon inoculation.

Materials and Methods

Two nursery experiments were carried out in 2003-05 at the Forest Nursery in Białe Błota Forest District in Bydgoszcz. The first two-year trial was conducted in 2003-04, and the second in 2004-05.

These experiments were run on a brown podzolic soil formed from loose sandy soil, in a *split-plot* system with four replications. Two different factors were compared. The first row factor – irrigation, was used in the three following treatments (main plots): without irrigation (control), drip irrigation, micro-jet sprinkling. The second row factor – fertilization, was used in the two variants (subplots): mineral fertilization (standard applied in forest nurseries) and organic fertilization (compost).

Seed origin and seeding density were similar to standard nursery practices [10]. Scots pine seeds were obtained from the seed stand of Forest District in Bydgoszcz. The plot area was 4 m^2 and contained 4 rows (4 m length) of Scots pine seedlings. Total number of plots in each experiment was 24 (3 x 2 x 4). Organic fertilizer was produced from sewage sludge (80%) and highmoor peat (20%). This fertilizer was spread in the spring (dose: 100 $t \cdot ha^{-1}$) and mixed with the topsoil (10 cm deep) before establishing the field experiments. Introduction of edaphon consisted of mixing topsoil (2 cm deep) with organic matter obtained from the surface of partial cutting in habitat of fresh coniferous forest. This substrate contained the living soil mesofauna which was very abundant. This measure was conducted directly before sowing every year. Mineral fertilization applied in the standard nursery treatment [8] was as follows: 70 kg $N \cdot ha^{-1}$, 70 kg $P \cdot ha^{-1}$, 80 kg $K \cdot ha^{-1}$ and 4 kg $Mg \cdot ha^{-1}$.

Drip irrigation was applied using "T-Tape" drip lines (inline emitters spaced 20 cm apart). Micro-jet irrigation was done with the use of "Hadar" micro-jets. Terms of irrigation and water rates were established according to "Directives for irrigation of forest nurseries on open areas" [11].

Climatic Conditions and Irrigation

Mean air temperature during the vegetation period (April-September) in the years of the study was 14.2°C, ranging from 13.4°C to 14.9°C, in 2004 and 2003, respectively (Table 1). Mean monthly values of air temperature in 2003-05 varied from 7.1°C in April to 18.3°C in July.

Total rainfall from 1 April to 30 September, on average for 2003-05, amounted to 208.3 mm, ranging from 177.5 mm to 244.5 mm, in 2003 and 2004, respectively. Among the months of the vegetation period, July was characterized by the highest rainfall amount (56.9 mm), and April by the lowest (16.4 mm).

Seasonal irrigation rates were dependent on rain-precipitation. The highest amounts of water were applied in case of the one-year-old seedling plantation in the vegetation period 2003 (Table 2). Total rates of water in drip

Table 1. Climatic conditions during the vegetation period.

Year	Months of vegetation period						IV-IX
	IV	V	VI	VII	VIII	IX	
Air temperatures (°C)							
2003	6.4	14.4	17.6	19.2	18.4	13.6	14.9
2004	7.5	11.3	14.7	16.4	17.9	12.7	13.4
2005	7.4	12.2	14.9	19.4	16.3	14.8	14.2
Mean for 2003 – 2005	7.1	12.6	15.7	18.3	17.5	13.7	14.2
Rainfall (mm)							
2003	13.3	12.1	34.3	88.8	17.8	11.2	177.5
2004	12.1	44.4	35.8	41.8	85.6	24.8	244.5
2005	23.8	69.5	30.7	40.2	20.9	17.9	203.0
Mean for 2003 – 2005	16.4	42.0	33.6	56.9	41.4	18.0	208.3

Table 2. Seasonal irrigation water rates used in the forest nursery of Scots pine (mm).

Year of the study	Drip irrigation	Microjet sprinkling
One-year old seedling plantation		
2003	210	310
2004	120	170
Mean for 2003 – 2004	165	240
Two-years old seedling plantation		
2004	51	70
2005	79	105
Mean for 2004 – 2005	65	87

irrigation and micro-jet sprinkling were higher in case of the one-year-old seedlings than those for the two-year old seedlings. It can be explained by the different water requirements of the Scots pine seedlings during their first and second seasons of growing [11].

Average seasonal irrigation rates equaled 240 mm in micro-jet sprinkling and 165 mm in drip irrigation in case of one-year-old seedlings, as well as 87 mm and 65 mm in the case of two-year-old seedlings, respectively.

Soil Analyses

The experiment was carried out on a brown podzolic soil which was characterized by the following soil profile: Ap-ABv-Bv-BvC-C. Samples were taken from top horizon (0-15 cm) of all the experimental plots. Soil samples were brought to the laboratory and air-dried at room temperature before being passed through a 1 mm sieve. The following chemical and physico-chemical properties were determined [12]:

- active acidity (pH in H_2O) was determined in hydrosuspension (soil/aqua destillata ratio as 1:2.5) by potentiometric method with the use of pH-meter CPC-551, (ELMETRON),
- exchangeable acidity (pH in KCl) was determined in suspension of 1M KCL (soil/KCl ratio as 1:2.5) by potentiometric method with the use of pH-meter CPC-551, (ELMETRON),
- organic carbon (C_{org}) was determined by Tiurin method, total nitrogen (N_{total}) by Kjeldahl method,
- available P and K by Egner-Riehm method, by extraction of samples in solution of Ca-lactate; P was determined in soil filtrate by colorimetric method, with the use of spectrophotometer MARCEL MEDIA, and K was determined in soil filtrate by emission spectrometry method with the use of spectrometer PU-9100 X, (Philips), available Mg was determined by Schachtschabel method, by extraction of soil samples in solution of 0.0125 M $CaCl_2 \cdot 6H_2O$, measurement of Mg content was determined by atomic absorption spectrometry, with the use of spectrometer PU-9100 X, (Philips),
- available microelements (Zn, Cu, Mn) were determined by atomic absorption spectrometry, with the use of spectrometer PU-9100 X, (Philips), after their previous extraction from the soil with the use of solution 1M HCl;
- granulometric composition of a soil was determined by areometric method of Bouyoucos in modification of Casagrande and Prószyński [12].

Growth and Mycorrhizal Analyses

In late autumn plant growth was evaluated. The height of seedlings (cm) and shoot diameter (mm) were measured.

The root systems of the seedlings were studied using a stereomicroscope (magnification 10-50x). To standardize the samples, lateral roots were taken from three different levels of root system of each (top, middle and bottom), cut into small pieces (ca. 1cm), and mixed in a Petri dish containing water. The percentage of mycorrhizal short roots for each plant was assessed by counting at least 200 short roots from the mixture of segmented roots under the stereomicroscope [13]. Following the guidelines of Agerer [14], and Ingleby et al. [15] morphological data were recorded on overall morphology and colour.

Sampling and Observations of Occurrence of Mites

To investigate the occurrence of mites, soil samples were taken twice a year (in May and October) in successive years. Samples of 17 cm^2 and 3 cm deep were taken from all plots in 3 replications (this mean 3 samples per plot). Mites were extracted from the material in high gradient Tullgren funnels. On average, 3034 mites (Acari) were identified to order, according to Hammen's systematics [16].

Statistical Evaluation

The experimental data was statistically processed by analysis of variance [17]. Fisher-Snedecor test was used to determine a significance of influence of experimental factors and Tukey test was used to define significant differences between the combinations. The data of mites were ln-transformed (x+1) prior to analyses [18].

Results and Discussion

Soil Characteristics

Soil pH in particular combinations was neutral and alkaline (Table 3). Soil pH values which were measured in H_2O and 1-molar KCl, ranged from 6.95 to 7.23 and from 6.70 to 7.09, respectively. In the treatment without organic fertilization it was found that the active acidity of a soil was decreased, more distinctly under drip irrigation than under micro-sprinkler irrigation, in comparison to control plots (without irrigation). Similar tendency was found in case of the exchangeable acidity. It can be connected with leaching of exchangeable cations. The lowest soil pH, both for active acidity as well as for exchangeable acidity, was found in case of treatment with micro-sprinkler irrigation. The highest pH values were found on control plots.

It was found that the content of organic carbon (C_{org}) was higher in samples from plots with organic fertilization than that from plots without compost. The lowest amounts

Table 3. Soil characteristics under different irrigation and fertilization systems.

Soil characteristics	Treatments					
	Control		Drip		Microjet	
	MF	OF	MF	OF	MF	OF
pH H_2O	7.23	7.26	6.95	7.11	7.15	7.05
pH KCl	7.06	7.09	6.70	6.90	6.86	6.84
$C_{org.}$%	1.23	1.86	1.15	1.77	1.30	1.91
N_{total}%	0.08	0.11	0.07	0.09	0.08	0.14
K_2O mg·$100g^{-1}$	2.18	1.15	1.91	1.62	2.06	1.45
P_2O_5 mg·$100g^{-1}$	4.69	2.84	4.11	3.08	3.54	2.56
Mg mg·$100g^{-1}$	2.55	1.42	2.42	1.10	1.89	1.22
Zn mg·kg^{-1}	0.30	0.33	0.42	0.31	0.39	0.42
Cu mg·kg^{-1}	0.22	0.15	0.25	0.18	0.22	0.15
Mn mg·kg^{-1}	17.44	12.24	18.55	10.38	14.26	11.25

MF, OF – without organic fertilization and with organic fertilization (compost), respectively

of C_{org} were found in drip-irrigated plots. Application of organic fertilization caused an increase of the total nitrogen content. The highest concentration of this element (0.14%) was noted in treatments with micro-sprinkler irrigation. No major differences of total nitrogen content were noted in combinations without organic fertilization. The content of available forms of macroelements ranged in case of P_2O_5 from 2.56 to 4.69 mg·$100g^{-1}$, for K_2O from 1.15 to 2.18 mg·$100g^{-1}$, and in the case of Mg from 1.10 to 2.55 mg·$100g^{-1}$. This means that soil fertility in terms of available forms of phosphorus and potassium, determined according to Egner-Riehm method, was very low [12]. Based on data included in Table 3, it was found that drip irrigation and micro-sprinkler irrigation caused the leaching of available forms of P and Mg in treatments without organic fertilization. Data concerning the content of available forms of Mg indicated that the amounts, under conditions of this experiment, ranged from a low to a medium level of a 5-degree scale [12].

Analyses of Zn, Cu and Mn indicated that the studied soils were characterized by a considerable shortage of these elements [12]. Application of organic fertilization did not cause an increase of the content of available forms of these elements.

Growth of Scots Pine Seedlings

Growth is the first process to be inhibited when sufficient water is lacking. Water in forest-tree nurseries is best regulated through carefully designed irrigation systems and practices [6]. Irrigation significantly increased the height of one-year old Scots pine seedlings from 8.9 cm on control plots to 13.2 and 13.7 cm for micro-jet sprinkling and drip irrigation, respectively (Table 4). In the case of seedlings during their second growing season, irrigation significantly increased the height from 26.4 to 33.6 and 34.8 cm for micro-jet sprinkling and drip irrigation, respectively. There were no significant differences in height between seedlings irrigated with drip system and micro-jets. However, seedlings grown on drip-irrigated plots were every year slightly taller than those cultivated under micro-jet sprinklers. These results are in agreement with those of several other studies [19, 20], which showed that reducing irrigation improves seedling growth. In experiment carried out by Hilszczańska [1], Scots pine seedlings grown on plots daily irrigated were shorter (7.2 cm) than those on plots irrigated once a week (8.3 cm). Results of the experiment conducted by Bergeron et al. [21] also indicate that nursery managers can reduce the quantity of irrigation water used without significantly affecting the growth or physiology of seedlings. On the other hand, irrigation by jet-type sprinklers contributes to spatial variability of substrate water content and growth of seedlings grown outdoors and maximum height growth and seedling biomass is attained when average seasonal substrate water content is approximately 40% (v/v) [22].

Use of organic fertilization (compost produced from sewage sludge and highmoor peat) significantly influenced seedling growth. Scots pine plants grown on plots fertilized with compost were, on average, 1.5 cm and 8.6 cm taller than those cultivated under control (without compost) in their first or second growing seasons, respectively.

A significant interaction of organic fertilization with irrigation was found. Irrigated seedlings grown on plots fertilized with compost (sewage sludge and highmoor peat) were taller than plants cultivated under control conditions. These results corroborate those of Lamhamedi and Gagnon [23], who showed that to produce quality seedlings, forest nursery irrigation and fertilization management must be optimized. In an experiment conducted by Heiskanen [24] – Scots pine seedlings growing in light *Sphagnum* peat growth medium were subjected to three irrigation treatments – in the wet irrigation treatment, height growth was lower than that in the other treatments. Rapid growth was obtained by irrigating at -5 to -10 kPa matric potential.

Irrigation significantly increased the Scots pine seedling diameter only during their first growing seasons. There were no significant differences between seedlings irrigated with drip system and those watered with micro-jets, except for a tendency of increased diameter of seedlings irrigated with drip line. Interaction between irrigation and organic fertilization in shaping of the seedling diameter occurred. Increased diameters were found on plots fertilized with compost and irrigated with micro-jets. In an experiment conducted by Fayle and Axelsson [25], daily irrigation alone increased Scots pine stem growth over the untreated condition but less than in treatment with fertilization.

Table 4. Influence of irrigation and fertilization on Scots pine seedling height and diameter.

Irrigation	Fertilization	One-year old seedling*		Two-year old seedling**	
		height (cm)	diameter (mm)	height (cm)	diameter (mm)
Control	MF	9.2	2.3	22.9	7.0
	OF	8.6	2.3	30.0	9.3
Drip	MF	12.2	3.0	30.2	7.5
	OF	15.2	3.1	39.4	9.0
Microjet	MF	12.1	2.8	29.0	7.7
	OF	14.4	3.0	38.4	8.3
Influence of irrigation (I)					
Control	-	8.9	2.3	26.4	8.2
Drip	-	13.7	3.0	34.8	8.3
Microjet	-	13.2	2.9	33.6	8.0
Influence of fertilization (II)					
-	MF	11.2	2.7	27.3	7.4
-	OF	12.7	2.8	35.9	8.9
LSD0.05	(I)	0.615	0.319	2.566	n.s.
	(II)	0.325	0.167	1.338	0.250
	(I) x (II)	n.s.	0.290	3.045	1.099
	(II) x (I)	0.733	0.379	2.318	0.433

MF, OF – see Table 3; *, ** – mean for 2003-04 and 2004-05, respectively

Mycorrhizal Colonization

The greatest percentage of mycorrhizas was noted for seedlings in treatment without watering and mineral fertilization (Fig. 1). The lowest percentage of mycorrhiza was observed in seedlings in the treatment with drip-watering and organic fertilization.

In the case of 2-year-old seedlings, the dominant morphotype belonged to *Thelephora terrestris* Ehrh. (Fig. 2). A similar percentage of these mycorrhizae were observed in all treatments, except the treatment irrigated with microjets and fertilized with mineral fertilization (M-MF). In this treatment mycorrhizae consisted of a similar percentage of *Laccaria laccata* (Scop.) Fr. and *Suillus* sp.

Results showed that the ectomycorrhizal community may vary with moisture conditions. The occurrence of *T. terrestris* ectomycorrhiza was found to be stimulated by lower soil moisture, although this species was generally frequent in all plots, irrespective of fertilization and soil moisture regime. These data are partly in agreement with the view that specific nursery conditions (high nutrition and moisture) increase development of mycorrhizal hyphae of *T. terrestris* [26, 27]. The ubiquitous character of *T. terrestris* makes it a strong competitor in nursery soil [28, 29].

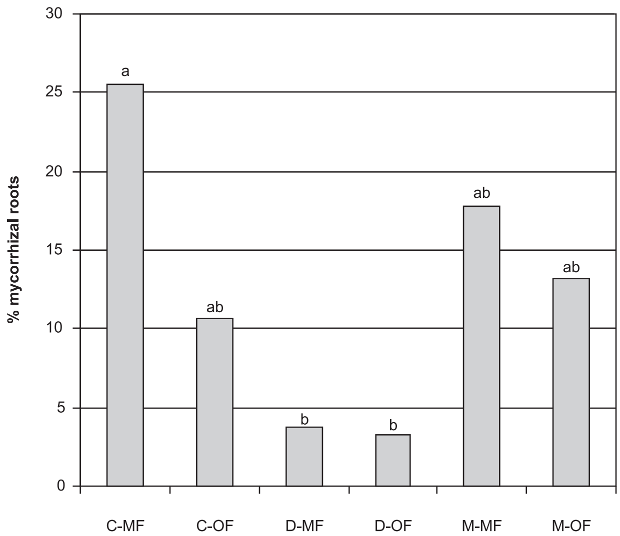

Fig. 1. Mycorrhizal colonization on Scots pine seedlings in different systems of watering and fertilization. C, D, M – non-irrigated (control) plots, drip-irrigated plots and micro-sprinkled plots, respectively; MF and OF – plots fertilized with mineral fertilizers and organic fertilizer (compost), respectively. Values followed by different letters are significantly different at $p< 0.05$, (n=5)

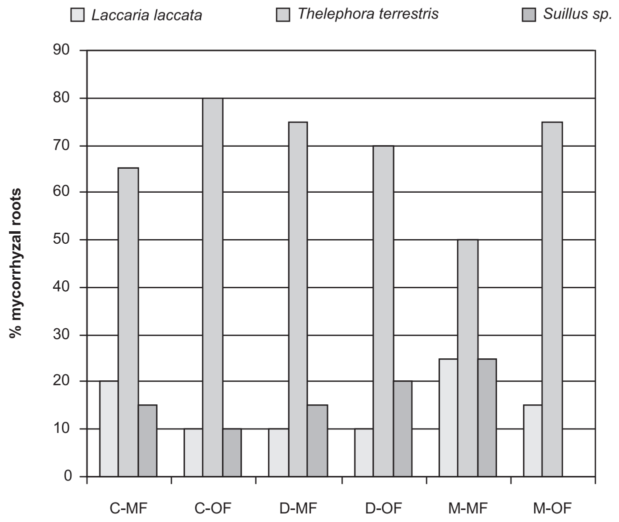

Fig 2. Frequencies of mycorrhizal morphotypes in different treatments of watering and fertilization on 2-year-old seedlings of Scots pine. Explanation, see Fig. 1.

T. terrestris, a representative of the medium-distance smooth exploration type, reduced the concentration of nitrogen, phosphorus and potassium in the fermentation horizon organic matter to a considerably lower degree than did *Suillus bovinus* (L.) O. Kuntze*,* a representative of the long-distance exploration type [30]. Hence, the presence of *T. terrestris* could explain why the seedlings in our experiment that had greater number of these mycorrhizas also had lower growth parameters.

Occurrence of Mites

The density of mites in soils of pine forests usually reaches several hundred thousand individuals per 1 m^2 of soil area [31]. Natural populations of Acari living in the same forest nursery (without the inoculation treatment) were described in our previous paper [32]. In the study area of the forest nursery, the density of mites in the plantation of Scots pine seedlings was low (2460 individuals$\cdot m^{-2}$). In a birch plantation (also without the inoculation treatment), the density of these microarthropods was over twice as high [33]. In both cases, natural populations of Actinedida were the predominant type of mites found in forest soils; Oribatida usually are dominant. These saprophages compose about 70% of all mites [31].

In this experiment, after the forest edaphon inoculation of the nursery soil, the average density of mites ranged from 2,090 to 12,870 individuals$\cdot m^{-2}$, depending on the experimental treatment (Table 5). On the basis of the analysis of variance (ANOVA), it was found that density of mites was significantly affected by irrigation. This influence was visible both in the first and the second year of the study. Organic fertilization did not significantly affect total density of these arthropods.

A significant increase in the density of Acari in irrigated plots, as compared to those without irrigation (control plots), was noted. The highest density of these arthropods was detected in the case of micro-jet sprinkling and organic fertilization. In the first year of the study, the density of mites was still distinctly differentiated and the positive effect of irrigation on mites was not visible in all plots. In the second year, an increased density of Acari was noted in the majority of plots.

In the area studied, Actinedida were usually the predominant order of soil mites – 56.4% of Acari. In the first year of the study their percentage was higher than in the second year. An opposite tendency was noted for oribatid mites – their percentage increased from 28 to 43.6% between the first and second years, respectively. Predacious Gamasida were the third most important group, in terms of abundance. Their percentage was on average 6.2% of all mites. In this experiment, mean density of Gamasida ranged from 190 to 400 individuals$\cdot m^{-2}$. For comparison, soils of pine forests are characterized by a considerably higher density of the mites – 7,800-24,100 individuals/m^2 [34]. In the adjacent forest tree nursery, on the Scots pine seedling plantation, an occurrence of Gamasida was not noted [32], and on the birch plantation the density of Gamasida was especially low – 100 individuals/m^2 [33]. Results indicated that the experimental treatments did not influence the abundance of Gamasida, and during the successive years their density was low. Remaining groups of mites (Acaridida, Tarsonemida) occurred sporadically.

From an ecological point of view it is interesting that in most plots during the second year of study, a significant increase of density of saprophage Oribatida was noted. This confirms that the edaphon inoculation was well-timed and that the groupings of mites were advantageously reconstructed and conformed to the structure that is characteristic of forest soils in which oribatid mites play a very important role. Literature confirms that these mites can feed on ectomycorrhizal fungi [35, 36]. In addition, soil fauna can influence fungal growth by grazing [37, 38], that may split the hyphal connections. Soil animals can also directly relocate nutrients by defecation, and transfer microbial propagules into fresh substrates [39, 40].

Statistical analysis indicated that irrigation was the only factor influencing the abundant increase of Oribatida. The increase in abundance of these mites as well as other soil micro-arthropods due to irrigation has been cited in literature [41, 42].

The scientific literature includes many reports about the bioindication value of oribatid mites [31]. Among soil fauna, saprophages are recognized as especially good bioindicators [43, 44]. Saprophage Oribatida mites are regarded, among others, as useful bioindicators of the degree of decomposition and biological properties of forest humus [45]. Therefore, an increase in their abundance in nursery soils may indicate that these soils are characterized by improved biological properties and a higher ecological balance which, in turn, can directly influence seedling production in a forest tree nursery.

Table 5. Abundance (N in 10^3 individuals m^{-2}) of mites under different irrigation and fertilization systems.

Group of mites	Year	Treatments						I	F
		Control		Drip		Microjet			
		MF	OF	MF	OF	MF	OF		
Acaridida	I	-	-	-	-	0.03	-	ns	ns
	II	0.03	-	0.08	0.08	0.05	-	ns	ns
	Avg	0.01	-	0.04	0.04	0.04	-	ns	ns
Actinedida	I	1.25	1.15	7.53(1)	1.05	4.01	10.99(1)	0.022	ns
	II	1.08	1.30	4.31(1)	3.46(1.2)	2.88(1)	6.22(1.2)	0.006	ns
	Avg	1.17	1.23	5.92(1)	2.26	3.45(1)	8.60(1)	<0.001	ns
Gamasida	I	0.25	0.25	0.58	0.33	0.28	0.48	ns	ns
	II	0.13	0.25	0.18	0.23	0.25	0.33	ns	ns
	Avg	0.19	0.25	0.38	0.28	0.26	0.40	ns	ns
Oribatida	I	1.20	0.70	1.30	0.98	1.53	1.25	ns	ns
	II	0.65	0.48	4.77(1.2)	4.29(1.2)	3.29	6.35(1.2)	<0.001	ns
	Avg	0.93	0.59	3.04(1)	2.63(1)	2.41(1)	3.80(1)	<0.001	ns
Tarsonemida	I	0.03	0.03	-	0.03	-	0.13	ns	ns
	II	-	0.03	-	0.08	-	-	ns	0.04
	Avg	0.01	0.03	-	0.05	-	0.06	ns	0.019
Acari total	I	2.73	2.13	9.41(1)	2.38	5.84	12.84(1)	0.003	ns
	II	1.88	2.06	9.33(1)	8.13(1.2)	6.47(1)	12.89(1)	<0.001	ns
	Avg	2.31	2.09	9.37(1)	5.25(1)	6.16(1)	12.87(1)	<0.001	ns

MF, OF – see Table 3
(1) – significant between control plot MF and a certain variants at $p < 0.05$; (2) – significant between I and II year, $p < 0.05$; F – fertilization effect (p); I – irrigation effect (p); Avg – mean

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