IOP Conference Series: Earth and Environmental Science

Biometric and Microbiological Approach to the aAsessment of the State of Young Plantations Hippophae Rhamnoides l. Subsp. Chinensis Rousi on Disturbed Highlands of Hongyuan County, Sichuan Province, China

Biometric and Microbiological Approach to the aAsessment of the State of Young Plantations Hippophae Rhamnoides l. Subsp. Chinensis Rousi on Disturbed Highlands of Hongyuan County, Sichuan Province, China

L A Lepeshkina[a], I V Cherepukhina[b], A A Voronin[c], Li Ling[d], M A Klevtsova[e]

Voronezh State University, Universitetskaya pl. 1, Voronezh, Russia, 394018

E-mail: [a]lilez1980@mail.ru, [b]icherepukhina@gmail.com, [c]voronin@bio.vsu.ru, [d]liling0616@foxmail.com, [e]klevtcova@geogr.vsu

Abstract. This research looks at the results of the assessment of the state of young plantations *Hippophae rhamnoides* L. subsp. *chinensis* Rousi on disturbed highlands of Hongyuan County using biometrical and microbiological approaches. It was found that the growth dynamics of young seedlings corresponds to the initial stages of ontogenesis, but has some features. Plantations of *H. rhamnoides* L. subsp. *chinensis* Rousi are classified as healthy with signs of weakening. The number of healthy plants is 60.5 %. Soils under plantations are characterized by high heterogeneity of microbiological conditions. The ratio of the main taxonomic and ecological-trophic groups of microorganisms indicates the poverty of the soil with available nitrogen. It is predicted that further these plantations will be able to perform environmental functions.

1. Introduction

Throughout the world, large areas of land are subject to desertification and degradation due to deforestation and overgrazing. As a result, centers of wind and water erosion are formed, soil fertility is reduced [1]. A special role in the ecological restoration of such areas is given to plants that grow rapidly in poor and disturbed lands, fix the mobile surface of the soil and increase their fertility [2]. Among these plants – *Hippophae rhamnoides* L., which is actinorhizal plant and forms a symbiotic relationship with nitrogen-fixing bacteria of the genus *Frankia* [3].

Sea-buckthorn (*H. rhamnoides* L. subsp. *chinensis* Rousi) is the dioecious and deciduous shrub widely distributed in the highlands (800-3600 m altitude) of north-west Sichuan [4]. Its plantings are widely used for the ecological restoration of disturbed lands: the restoration of industrial waste dumps, for the purposes of agroforestry and reforestation.

In China, the area of land exposed to desertification is 334 000 km^2 [1]. Soil degradation process is particularly active in the north-west of Sichuan Province. In order to stabilize the situation and preserve the highly productive pastures in the subalpine cold zone of meadows, the sea-buckthorn plantations are created.

The aim of this work is to study the assessment of state of young plantations of *H. rhamnoides* L. subsp. *chinensis* Rousi in disturbed highlands of Hongyuan County. Objectives of the study: to assess the vital state of plantations on biometric indicators of plants; to study the diversity of soil microorganisms and features of microbiological processes within the rhizosphere of plants.

2. Equipment and devices used in studies

Field research was conducted in early August 2018 by the staff of the Voronezh State University and the Sichuan Provincial Institute of Natural Resources Sciences. The study area is Hongyuan County in the east of the Tibetan Plateau, in the north-west of Sichuan, China. The territory belongs to the Yellow River basin.

The climate is continental monsoon cold-temperate zone of the plateau, there are no obvious boundaries of the four seasons. The average annual air temperature is 1.4ºC, extremely low - 36.0ºC, extremely high + 26.0ºC. The daily amplitude of air temperature is on average 16.3ºC. The average annual rainfall is 749.1 mm. The following climate characteristics are particularly notable: rarefied air, reduced atmospheric pressure, low oxygen content in the atmosphere, low dust and humidity, high intensity of solar radiation (more than 3000 hours of solar insolation per year). In general, the climate can be attributed to the semi-arid and humid type [5].

In the explored territory grass and shrubby communities on alpine and subalpine marsh, meadow and desert soils are presented [6].

Experimental young sea-buckthorn plantings were created during 2015-2018 on the hilly plateau 3459 m altitude, coordinates: 31 °10.670", 102 °37.572". About 200 000 seedlings of sea-buckthorn have been planted.

The size of the experimental territory of cultivation of sea-buckthorn is 70.5 hectares. The relief is largely wavy. There are sites with the dry and humidified soils. For more damp soils processes of gleying are noted. Certain sites with a bias more 5º have a mobile sandy surface. In decreases stagnation of water and processes of salinization is noted.

The dominating associations are various cereal communities with high participation of *Dasiphora fruticosa* (L.) Rydb., *Elymus nutans* Griseb. и *Artemisa sp.* In the composition of the flora are noted: *Rarunculus tanguticus* (Maxim.) Ovcz., *Sedum costantinii* Hamet, *Thlaspi arvensis, Galium verum, Peucedanum praeruptorum* Dunn., *Heracleum millefolium* Diels, *Gentiana sino-ornata* Balf. f., *Kobresia kansuensis* Kiikenth, *K. persica* Kük. & Bornm., *Aconitum pendulum* Busch, *Oxytropis kansuensis* Bunge, *Saussurea salicifolia* (L.) DC., *Polygonum viviparum* L. and other.

For assessment of a vital condition of sea-buckthorn plantations the biometric indicators of plants have been used (diameter of shoot at the surface of the soil, the shoot height, leaf length and width, number of side skeletal twigs, root depth, depth of roots nodules formation, their number and the size) received from not less than 100 plants on three trial sites.

According to the 3-point scale of the vital state of shrubby plants, the ratio of healthy seedlings without signs of weakening (1 point), weakened (2 points) and strongly weakened (3 points) was revealed. Signs of a weakened plant are a dry top of the shoot, yellowing of not more than 20% of the leaves, a weak increase in the main shoot. Signs of a severely weakened seedling are the dying off of the main shoot, yellowing of more than 20% of the leaves, drying out of the main skeletal twigs.

Six samples of the soil on which sea-buckthorn plantations are formed have been investigated. They were selected both directly in the rhizosphere of plants and in the surrounding soil. Soil moisture, pH, content of mobile forms of nitrogen (nitrate and ammonium) were determined by conventional methods. Accounting of number of microorganisms of various physiological, taxonomical and ecologo-trophic groups was carried out by method of seeding of soil suspension of different extent of dilution on elective nutrient mediums [7, 8].

The number of ammonifiers was considered on the meat-peptone agar, the microorganisms using mineral nitrogen – on the starch-ammoniac agar, oligoazophils – on the Eshbi culture medium. Cellulolytic microorganisms allocated on Vinogradsky's culture medium. The autochthonic group of microorganisms was defined on the nitrite agar, microflora involved in the synthesis of humus – a calculation method. The number of spore-forming bacterias – on meat-peptone a mash. Quantity of phosphobacteria – on Menkina's culture medium, micromycetes – on the acidified Capek's culture medium [8, 9].

3. Results and Discussion

The dynamics of growth and development of seedlings 1-3 years according to the main parameters corresponds to the initial ontogenetic stages. The main biometric characteristics of the plants *H. rhamnoides* subsp. chinensis is presented in table 1.

Table 1. Biometrical characteristics of plants *H. rhamnoides* subsp. *chinensis* in the field experiment.

Average							
Diameter of shoot at the ground surface	**Height of shoot, cm**	**Leaf length, cm**	**Leaf width, cm**	**Root depth, cm**	**Number of skeletal twigs**	**Depth of roots nodules formation, cm**	**Number of nodules / size of nodules, cm**
First year in the field experiment							
0.46	35.04	2.45	0.45	34.4	23.8	14.73	5.6/0.75
Second year in the field experiment							
0.93	54.5	2.85	0.58	54.8	30.5	24.7	9.5/1.63
Third year in the field experiment							
1.25	63.9	3.16	0.68	49.6	12.6	18.53	9.0/1.63

By the third year of sea-buckthorn seedlings naturally increases the diameter of the shoot (from 0.46 to 1.25 cm), its height (from 35.04 to 63.9 cm), length (from 2.45 to 3.16 cm) and width (from 0.45 to 0.68 cm) of the leaf, root depth (from 34.4 cm to 49.6 cm).

In the first and second year of development of seedlings the number of side twigs can exceed 30 pieces. Seedlings of the third year have already smaller number of twigs (less than 20) – 2-3 main skeletal axes are formed that is characteristic of a pregenerative stage of ontogeny of *H. rhamnoides*. Depth of roots nodules formation on the main root at first increases (to 24.7 cm), and for the third year begins to decrease and averages 18.53 cm. As a result of field researches several sea-buckthorn seedlings of the second year with high vitality have been found, but 43-55% of their roots nodules were the dead at a depth more than 20 cm (the size – 1.5-1.8 cm). Process of formation of consortia connections of *H. rhamnoides* L. subsp. *chinensis* with nitrogen-fixing bacteria quickly develops in the first and second year when the number of nodules on average reaches 9.5 pieces on one plant, and their average size is 1.63 cm.

It is established that in conditions of loose and mobile sandy soils of highlands Hongyuan County planted in early spring sea-buckthorn seedlings next year form a well-developed horizontal roots length 9.9 – 14.2 cm For the second and third year, the diameter of the main shoot reaches 55-65 cm, slows the growth of the main root, quickly start to grow horizontal, threadlike roots (longer than 40 cm). Active formation of nodules on such roots begins only in the third year.

The main fall-off of plants occurs in the first year after planting, when 50% of seedlings die. It was found that in the first year 37.2% of seedlings observed complete death of the shoot (40.6 cm). In 12.8% of seedlings showed signs of death, yellowing of leaves and drying of sleeping buds. They have not formed a symbiotic relationship with nitrogen-fixing bacteria (no nodules), the length of their root reaches 24.6-28.3 cm.

On a vital condition of the third year seedlings the following ratio (fig. 1) is received: healthy plants without signs of easing – 60.5%; weakened – 27.2%; strongly weakened – 12.3%.

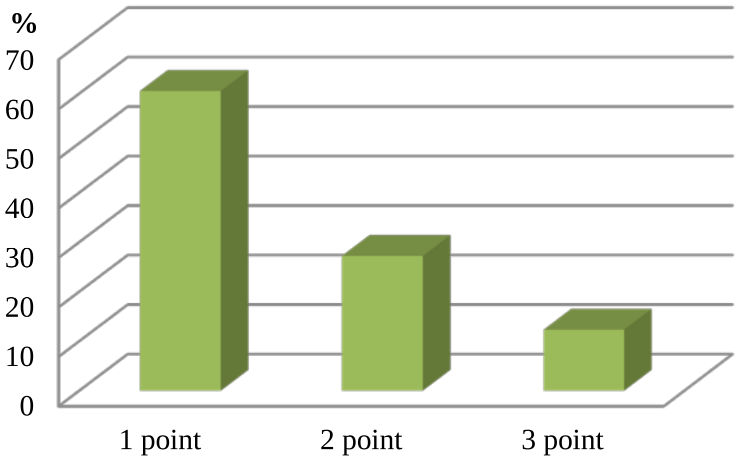


Figure 1. A ratio healthy (1 point), weakened (2 point) and strongly weakened (3 point) plants in sea-buckthorn plantations of the third year.

Under normal conditions, adventitious roots of sea buckthorn seedlings are actively developing in the second – third year [10]. This feature is very important for fixing loose and mobile sandy soils. It was found that in the experimental areas of the highlands of Hongyuan County, even in the third year, the seedlings did not form root offspring on horizontal roots.

The beginning of the generative stage of development in the third or fourth year of life [link] is characteristic of *H. rhamnoides*. Generative plants were not found in the studied plantings, which indicate the peculiarities of ontogenesis in these conditions.

Soil under sea-buckthorn plantations. The humidity of the soil of the explored territory varies over a wide range: from 8.9 to 15.8%. Higher humidity is noted in a rhizosphere of plants (tab. 2). Values pH soils on different sites varied from 5.41 to 6.58.

Table 2. Characteristic of soil conditions.

№ soil sample	pH	NO_3, mg/100 g of soil	N-NH_4, mg/100 g of soil	Soil humidity, %
1	5.65	1.68	7.98	8.9
2	5.76	1.81	7.64	12.8
3 (*rhizosphere*)	5.41	1.26	7.54	13.9
4	6.58	0.92	7.78	11.2
5	6.58	1.15	7.39	15.7
6 (*rhizosphere*)	6.45	0.30	7.99	15.8

The content of ammonium nitrogen in the soil is within 7.39-7.99 mg / 100 g of soil. The content of nitrate nitrogen in the soil is 1.15-1.81 and 0.92-1.68 mg/100 g of soil, respectively. In the rhizosphere of seedlings of the first year of life, it is naturally reduced to 1.26 mg/100 g of soil, in the rhizosphere of seedlings of the third year – to 0.30 mg/100 g of soil.

The number of ammonifiers bacteria in the studied soil varies in a wide range of values from 0.9 to 6.9 (fig. 2). More stable values are indicators for bacteria-immobilizers – from 2.5 to 10.1 CFU in 1 g of soil. The data obtained confirm the high heterogeneity of microbiological conditions and the predominance of the process of immobilization of nitrogen in the fraction of soil humus.

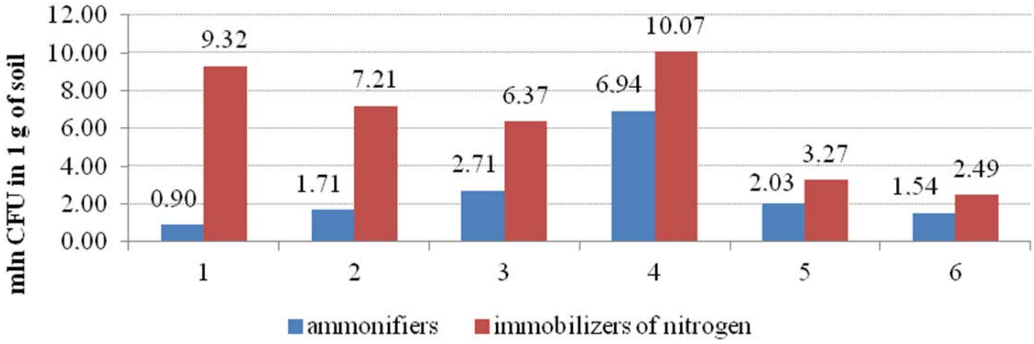


Figure 2. The number of ammonifiers and immobilizers in soil and the rhizosphere, million CFU in 1 g of soil (1-6 numbers of soil samples).

The presence of zymogenic microorganisms in the soil in an amount from 4.0 to 17.0. million CFU in 1 g of soil (fig. 3) indicates the process of increasing organic matter. And in almost all samples, this group of bacteria exceeds the number of autochthonic microflora.

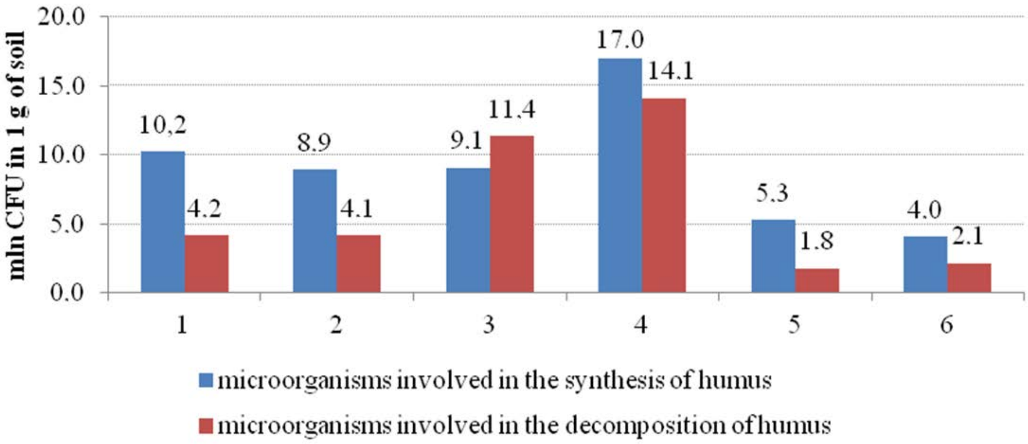


Figure 3. The number zymogenic (involved in the synthesis of humus) and autochthonic (involved in the decomposition of humus) microfloras in the soil and the rhizosphere, million CFU in 1 g of soil (1-6 numbers of soil samples).

The number of soil micromycetes is presented in the range of values from 15.2 to 47.1 thousand CFU in 1 g of soil (fig. 4), that can demonstrate existence in the soil micromycetes which participates in decomposition of various organic compounds. A part of products of this reaction will be used for synthesis of humus substances, another is used for food of plants.

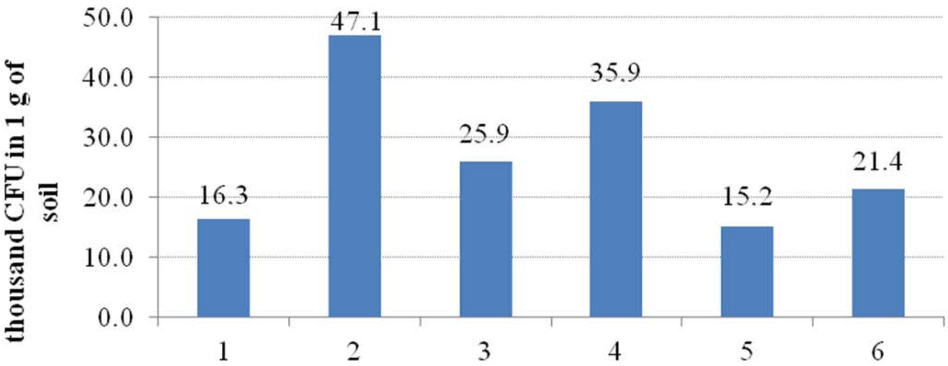
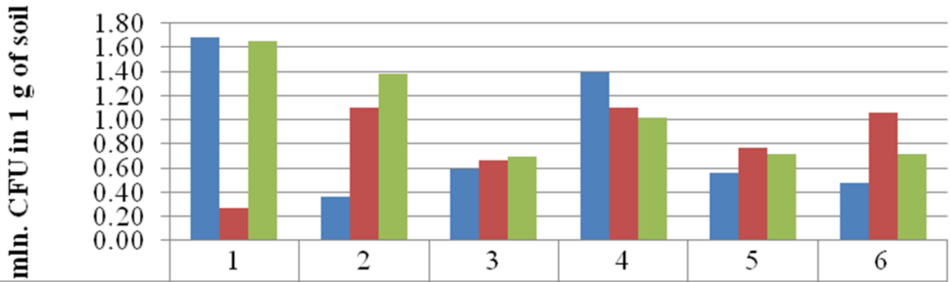


Figure 4. The number of micromycetes in the soil, thousand CFU in 1 g of soil (1-6 soil sample numbers).

The ratio of the main taxonomic groups of microorganisms in soil samples №5 and №6 is dominated by spore-forming bacteria (0.77; 1.06 mln. CFU in 1 g of soil), in samples №2, №3-cellulolytics (1.38; 0.70 mln. CFU in 1 g of soil), №1, №4-actinobacteria (1.68; 1.39 mln. CFU in 1 g of soil) (fig. 5).



	1	2	3	4	5	6
actinobacteria	1.68	0.37	0.59	1.39	0.56	0.47
spore-forming bacteria	0.27	1.10	0.66	1.10	0.77	1.06
cellulolytic microorganisms	1.65	1.38	0.70	1.01	0.71	0.71

■ actinobacteria ■ spore-forming bacteria ■ cellulolytic microorganisms

Figure 5. The number of microorganisms involved in the destruction of complex polymeric compounds in the soil, million CFU in 1 g of soil (1-6 numbers of soil samples).

In the studied soil among the groups of microorganisms involved in the cycling of nitrogen and phosphorus is dominated by oligoazophils, which develop on poor nitrogen substrate (fig. 6). This is followed by phosphobacteria, the number of which was higher in the soil and rhizosphere of the plant third years – 2.14-4.04 mln. CFU in 1 g of soil.

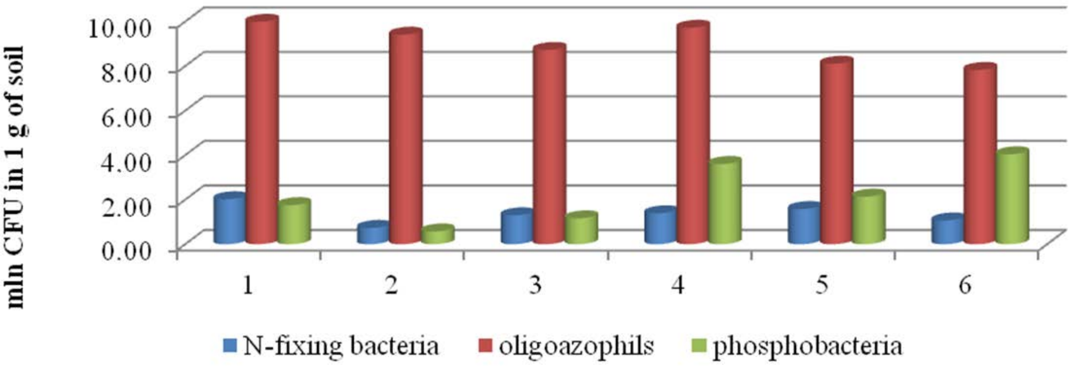


Figure 6. The number of microorganisms involved in the transformation of nitrogen and phosphorus compounds in the soil, million CFU in 1 g of soil (1-6 numbers of soil samples).

Values of number of diazotrophs (N-fixing bacteria) in all soil samples was at one level. This ratio of microorganisms shows the poverty of the substrate with available nitrogen, but the presence of phosphobacteria in the studied soils confirms their good provision with phosphorus.

4. Conclusion

According to the main biometric characteristics of plants, a positive dynamics in the development of sea buckthorn plantations of the first-third year of life in the disturbed lands of Hongyuan County was revealed. Young three-year plantations are classified as healthy with signs of weakening, where the number of healthy plants – 60.5%, weakened – 27.2%, severely weakened – 12.3%. It is revealed that third year seedlings do not form root offspring on horizontal roots and do not enter the generative stage of ontogenesis, which is due to more extreme conditions of the highlands environment.

Soils under stands of sea-buckthorn are characterized by a high heterogeneity of microbiological conditions and the dominance of the process of immobilization of nitrogen in the fraction of soil humus. For them domination zymogenic of microorganisms (4.0-17.0 million in1 g of soil) and a high share of micromycetes (47.1 thousand CFU in 1 g of soil). The ratio of the main taxonomic and ecologo-trophic groups of microflora indicates the poverty of the soil with available nitrogen.

According to the results of the study, it is predicted that young plantations of *H. rhamnoides* L. subsp. *chinensis* will be able to perform important environmental functions, consolidate and retain the mobile surface of sandy soils, prevent the development of deflationary and water erosion processes, control the loss of organic matter and maintain soil fertility.

References

[1] Kairis O Kosmas C Karavitis C (...) Zhonging W Ziogas A 2013 Evaluation and Selection of Indicators for Land Degradation and Desertification Monitoring: Types of Degradation, Causes, and Implications for Management (Environmental Management 54(5)) pp 971-982
[2] Li Y Li Z Wang Z (...) Jia Y Tian S 2017 Impacts of artificially planted vegetation on the ecological restoration of movable sand dunes in the Mugetan Desert, northeastern Qinghai-Tibet Plateau (International Journal of Sediment Research 32(2)) pp 277-287
[3] Zhou X Tian L Zhang J (...) Li X Tian C 2017 Rhizospheric fungi and their link with the nitrogen-fixing Frankia harbored in host plant Hippophae rhamnoides L (Journal of Basic Microbiology 57(12)) pp 1055-1064
[4] Li C Xu G Zang R Korpelainen H Berninger F 2007 Sex-related differences in leaf morphological and physiological responses in Hippophae rhamnoides along an altitudinal gradient (Tree Physiology 27(3)) pp 399-406
[5] Liu Y Bao Q Duan A Qian Z Wu G 2007 Recent progress in the impact of the Tibetan Plateau on climate in China (Advances in Atmospheric Sciences 24 (6)) pp 1060-1076

[6] Zhang R Wang Z Han G (...) Wu Q Gu C 2018 Grazing induced changes in plant diversity is a critical factor controlling grassland productivity in the Desert Steppe, Northern China (Agriculture, Ecosystems and Environment 265) pp 73-83
[7] Zhadambaa N Zenova G M Manucharova N A Shil'nikova V K 2003 Population dynamics of rare species of actinomycetes in the steppified desert soil of Mongolia (Eurasian Soil Science 36(10)) pp 1101-1104
[8] Dobrovol'skaya T G Zvyagintsev D G Chernov I Y (...) Stepanov A L Umarov M M 2015 The role of microorganisms in the ecological functions of soils (Eurasian Soil Science 48(9)) pp 959-967
[9] Zvyagintsev D G Zenova G M Sudnitsyn I I (...) Napol'skaya K R Belousova M A 2009 Dynamics of spore germination and mycelial growth of streptomycetes under low humidity conditions (Microbiology 78(4)) pp 440-444
[10] Yao J-H Li W 2013 Morphological and anatomical features during the formation of adventitious roots of sea buckthorn by micro-cuttage multiplication (Beijing Linye Daxue Xuebao/Journal of Beijing Forestry University 35(2)) pp 130-133