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EFFECTIVENESS OF GROWTH STIMULANTS IN THE CULTIVATION OF KOREAN RED PINE SEEDLINGS (*PINUS DENSIFLORA* SIEBOLD ET ZUCC.) IN THE PRIMORSKY KRAI CONDITIONS

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ABSTRACT

The influence of root-feeding stimulants Crezacin, Ribav-Extra, Zircon, Ecopin, Epin-Extra on the growth of Korean red pine seedlings (*Pinus densiflora* Siebold et Zucc.) at growing planting material is studied. Higher growth activity was observed in seedlings fed with growth stimulants Crezacin, Ribav-Extra and Ecopin at solution concentrations of 1ml/5l and 1ml/10l. It was revealed that these stimulants increase the growth of seedlings in height, neck diameter and root lobe length, and biomass. In the second year of seedlings growth, the laying of buds of side shoots was noted. Biennial seedlings, when using these stimulants in terms of growth, meet the requirements of the current OST 56-98-93. They can be used for planting on the forest area and transplanting into the school nursery department, for further cultivation of seedlings.

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1. INTRODUCTION

Primorsky Krai is one of the largest Far Eastern regions of the Russian Federation (Petropavlovsky, 2004). According to the latest accounting of the forest fund, the total area of forests of the region is 10295.2 thousand hectares. 5341.5 thousand hectares (51.7 %) of this area is occupied by coniferous trees. There are many valuable species of trees, shrubs, and lianas, which are not found anywhere else on the territory of the Russian Federation. In the forest fund representatives of the family “Pine-*Pinaceae* Lindl.” grow everywhere, which is represented by four genera: pine (*Pinus* L.), fir (*Abies* Mill.), spruce (*Picea* A. Dietr.) and larch (*Larix* Mill.).

In the southern districts of the region (Khasansky, Shkotovsky, and Vladivostok), there is natural growth of Korean red pine (*Pinus densiflora* Siebold et Zucc.), occupying a small area – about 4.0

thousand hectares (Koryakin, 2010; Urusov et al., 2004; Urusov et al., 2007).

Korean red pine is an endangered species of the Russian Far East (Urusov et al., 2004; Urusov et al., 2007). It is listed in the Red books of Primorsky Krai and the Russian Federation (Golovanov et al., 1988; Red book of Primorsky Krai: Plants, 2008).

It occurs in separate preserved sporadic areas located on hard-to-reach massifs. The natural habitat of Korean red pine is mountain forests. In the conditions of mountain relief, vulnerable and difficult-to-restore ecological systems, this tree species is one of the best forest-reclamation species for fixing ravines, slopes, sands, protective and roadside strips. It is used in landscaping. However, seed years in the pine forests of the region are repeated in three to four years (Urusov et al., 2004; Urusov et al., 2007; Usenko, 1969). During such periods of storage, seeds reduce germination energy and germination. The use of growth stimulants (regulators), which proved themselves positively in agriculture, can improve the sowing quality of seeds and ensure the restoration of this valuable tree species. Many studies conducted in different forest conditions confirm their prospects. Laboratory and soil germination of seeds are increased. Seedlings grow vigorously. Their safety is high (Galdina & Shevchenko, 2012; Kirienko & Goncharova, 2016; Nikitenko et al., 2005; Ostroshenko & Ostroshenko, 2010; Ostroshenko et al., 2014; Ostroshenko et al., 2015; Ostroshenko, 2014; Pentelkin, 2001; Pentelkina, 2002; Pentelkina & Ostroshenko, 2005).

This work is devoted to the study of the effectiveness of growth stimulants (regulators) of natural (Ribav-Extra, Zircon, Ecopin) and synthetic (Crezacin, Epin-Extra) origin in the growth of Korean red pine seedlings. These growth stimulants have proven themselves positively in agriculture. The research was initiated on the possibility of their application in forestry, particularly in forest restoration [1, 3, 9-11]. (Galdina & Shevchenko, 2012; Kirienko & Goncharova, 2016; Ostroshenko et al., 2014; Ostroshenko et al., 2015; Ostroshenko, 2014).

Crezacin –synthetic adaptogen and immunostimulant triethanolammonium salt of orthocresolacetic acid, $C_{15}H_{25}NO_6$ is characterized by a wide range of biological activity. The drug is easily soluble in water and alcohol, insoluble in ether.

Ribav-Extra is a product of the vital activity of mycorrhizal fungi isolated from ginseng roots by biotechnology and contains a unique natural complex (amino acids, phytohormones, vitamins), which in negligible doses activates all processes of vital activity of plants. Active ingredient of Ribav-Extra: 0.00125 g / l L-alanine + 0.00196 g / l L-glutamic acid.

Growth stimulant Zircon is produced on the basis of purple coneflower and contains esters based on hydroxycinnamic acids dissolved in alcohol, namely, caffeic acid and its derivatives: chicory and chlorogenic acids, which perform the function of the active principle in this preparation and determine its profound effect on plant cell metabolism, participation in the regulation of hormonal status and enzymatic profile (Malevannaya & Bykhovskaya, 2001).

Biological preparation Ecopin is the growth stimulant of natural origin. It consists of 6.2 g/kg of poly-beta-hydroxybutyric acid + terpenic acids + set of nutrients and is a concentrated product of biosynthesis of beneficial soil bacteria + starting set of nutrients. It is a universal biostimulant of plant growth and development, which has an anti-stress effect. The basis of this biological preparation contains a concentrated product of biosynthesis of beneficial soil bacteria and a starting set of nutrients.

Growth stimulator Epin-Extra - a synthetic analog of a natural plant hormone. The active substance of the drug is epibrassinolid, belongs to the class of brassinosteroids, natural plant

hormones. The mechanism of its action is to activate the plants' own phytohormones. It is epibrassinolide that causes the activation of biological processes in plants, literally saving them from diseases, old age and at the time of stress.

In general, the preparations used for the experiments are low-toxic, do not have a mutagenic effect, they are recommended for pre-sowing, root and foliar feeding of plants. These growth stimulants increase sprouting and germination of seeds, increase yields. They are safe for humans, warm-blooded animals, and beneficial insects, practically not dangerous for fish. They reduce the content of heavy metal salts in plants; increase resistance to frost, drought, excessive waterlogging. They are easily soluble in water, do not accumulate in the soil, do not pollute ground and surface water, not phytotoxic and environmentally friendly. The preparations are included in the "List of pesticides and agrochemicals permitted for use in the territory of the Russian Federation" (RMOA, 2017).

The purpose of this work is to study the effect of root fertilization of one-two-year seedlings of Korean red pine (*Pinus densiflora* Siebold et Zucc.) with growth stimulants Crezacin, Ribav-Extra, Zircon, Ecopin, Epin-Extra on their growth and development. Based on this purpose, the following tasks were solved:

1. Analysis of forest conditions of the object of works;
2. Harvesting of Korean red pine seeds and their sowing in the nursery;
3. Conducting root fertilizing of one-year-old seedlings with solutions of growth stimulators of Crezacin, Ribav-Extra, Zircon, Ecopin, Epin-Extra;
4. Agrotechnical care and the subsequent monitoring of seedlings growth by height, root system, and phytomass in the course of two years;
5. Analysis of the influence of these growth stimulants on the cultivation of planting material.

2. WORKING PROCEDURE

The object of research is the nursery of the MTS-branch of the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. Seeds of a Korean red pine were collected in the woodland adjacent to the territory of the station in autumn, and in spring they are sown in beds of the nursery.

Preparation of the soil consisted of preliminary manual digging of the soil and the arrangement of ridges for seeds sowing. The height of the ridges was 20 cm from the soil surface. The location of the sowing rows in the beds was transverse. The distance between the centers of the sowing lines was 20 cm, between the variants of experiments – 40 cm. The seeds were soaked in KMnO₄ solution before sowing. The depth of sealing was 1.5 cm.

After germination and the beginning of growing season of the seedlings, then in two weeks of the first year of growth and the beginning of the growing season of the second year of growth (in June), root fertilizing of seedlings with fresh solutions of stimulators was carried out: Crezacin, Ribav-Extra, Zircon, Ecopin, and Epin-Extra. Feeding was carried out in the evening, in dry weather, in the absence of a forecast for rain. Solution concentrations: 1 ml / 5l and 1 ml/10l of water. Control – seedlings did not have root feeding with growth stimulants.

Within two years, the seedlings were carried out regular agrotechnical care, consisting of

weeding and loosening the soil between the sowing lines: in the first year of growth of seedlings – twice, in the second year – once.

The seedlings were regularly watered. Their growth and condition were watched. At the end of the growing season of seedlings, from each variant of the experiment, by random sampling (every fifth seedling), 25 pieces of plants were selected (to ensure a small sample during statistical processing), in which the height of the above-ground part was measured. Average values and model specimens were calculated.

At the end of the growing season, three model seedlings of medium height were dug from each variant of the experiment. The roots of the plants were washed from the substrate, then wiped with a cotton cloth and dried in a shaded room. The length of the root lobe was measured with a ruler in the seedlings selected for the experiments. The diameter of the root neck was measured with a caliper to an accuracy of 0.1 mm. Seedlings were divided into the root system and the aboveground part (trunk, pine needles), dried, weighed on the scales of VLKT-500 to an accuracy of 0.01 g and these growth indicators were determined in the air-dry state.

The materials of the field experiments were statistically processed in the application program Microsoft Excel 2007. The results were compared with the variants of the experiment and with the control. The materiality of differences with control was calculated by Student's t-test (Doev, 2001).

Table 1: The impact of root-feeding stimulants on the growth of one-year Korean red pine seedlings (*Pinus densiflora* Siebold et Zucc.)

№	Growth stimulant / Solution concentration, ml/l	The average value of the height, $M \pm m$, cm	Materiality of differences	Diameter of the root neck, mm	Length of the lobe root, cm
1	2	3	4	5	6
1.	Control	$7,8 \pm 0,2$	-	2,5	6,9
2.	Crezacin				
	1×5	$9,1 \pm 0,2$	$4,6 \geq 3$	2,9	8,1
	Percentage to the control	+16,7		+16,0	+17,4
	1×10	$10,1 \pm 0,1$	$10,5 \geq 3$	3,0	8,6
	Percentage to the control	+29,5		+20,0	+24,6
3.	Ribav-Extra				
	1×5	$10,8 \pm 0,3$	$8,3 \geq 3$	3,2	7,4
	Percentage to the control	+38,5		+28,0	+7,2
	1×10	$11,4 \pm 0,2$	$12,9 \geq 3$	3,3	9,1
	Percentage to the control	+46,2		+32,0	+31,9
4.	Zircon				
	1×5	$8,2 \pm 0,1$	$1,8 \leq 3$	2,7	7,1
	Percentage to the control	+5,1		+8,0	+2,9
	1×10	$8,4 \pm 0,1$	$2,7 \leq 3$	2,9	7,2
	Percentage to the control	+7,7		+16,0	+4,3
	Ecopin				
	1×5	$9,7 \pm 0,2$	$6,8 \geq 3$	3,0	8,3
	Percentage to the control	+24,4		+20,0	+20,3
	1×10	$12,3 \pm 0,4$	$10,0 \geq 3$	3,4	8,6
	Percentage to the control	+57,7		+36,0	+24,6
5.	Epin-Extra				
	1×5	$8,8 \pm 0,2$	$3,6 \geq 3$	2,8	7,4
	Percentage to the control	+12,8		+12,0	+7,2
	1×10	$8,3 \pm 0,3$	$1,4 \leq 3$	2,6	7,3
	Percentage to the control	+6,4		+4,0	+5,8

3. RESULTS

The analysis of the data of meteorological observations carried out at the object of research

shows that the weather conditions during the experiments were within the average long-term.

The positive effect of root fertilizing with stimulants on the growth of experienced seedlings of Korean red pine was noted in the first year of growth. There was an increase in the root system. Thus, when feeding with the stimulant Crezacin, the average values for the length of the root lobe exceeded the control by 17.4-24.6 %, Ribav-Extra by 7.2-31.9 %, Ecopin by 20.3-24.6 %. Stimulants Zircon and Epin-Extra were less effective, exceeding the control by 2.9-4.3 % and 5.8-7.2 %. Accordingly, by the diameter of the root neck, they exceeded the control by 4.0-36.0 %, height 5.1-57.7 %. When using drugs of CrezaCin, Ribav-Extra and Ecopin differences from control were significant (Table 1).

These growth stimulants showed high efficiency in the second year of growth of seedlings. Drugs Crezacin, Ribav-Extra, and Ecopin had a positive effect on the increase in the length of the root lobe, exceeding the control, depending on the concentration of the solution by 21.1-27.5 %, 27.5-29.6 % and 2.8-21.1%. Stimulants Zircon and Epin-Extra had a less positive value. The excess to control was 10.6-13.4% and 4.2-6.3%. By the diameter of the neck root, the excess to the control was in the range of 2.6 to 18.4 %.

All tested stimulants had a positive impact on the growth of seedlings in height, exceeding the control by 15.1-44.3 %. Differences with control were significant (Table 2).

Table 2: The impact of root-feeding stimulants on the growth of two-year-old Korean red pine seedlings (*Pinus densiflora* Siebold et Zucc.)

№	Growth stimulant / Solution concentration, ml/l	The average value of the height, M±m, cm	Materiality of differences	Diameter of the root neck, mm	Length of the lobe root, cm
1	2	3	4	5	6
1.	Control	18,5 ± 0,4	-	3,8	14,2
2.	Crezacin				
	1×5	24,4 ± 0,6	8,2 ≥ 3	4,0	17,2
	Percentage to the control	+31,9		+5,3	+21,1
	1×10	25,1 ± 0,7	8,1 ≥ 3	4,3	18,1
	Percentage to the control	+35,7		+13,2	+27,5
3.	Ribav-Extra				
	1×5	26,7 ± 0,7	10,1 ≥ 3	4,5	18,4
	Percentage to the control	+44,3		+18,4	+29,6
	1×10	25,9 ± 0,7	9,1 ≥ 3	4,2	18,1
	Percentage to the control	+40,0		+10,5	+27,5
4.	Zircon				
	1×5	22,4 ± 0,6	5,4 ≥ 3	4,1	15,7
	Percentage to the control	+21,1		+7,9	+10,6
	1×10	23,9 ± 0,4	9,5 ≥ 3	4,3	16,1
	Percentage to the control	+29,2		+13,2	+13,4
	Ecopin				
	1×5	21,8 ± 0,5	5,2 ≥ 3	4,0	14,6
	Percentage to the control	+17,8		+5,3	+2,8
	1×10	24,6 ± 0,6	8,5 ≥ 3	4,2	17,2
	Percentage to the control	+33,0		+10,5	+21,1
5.	Epin-Extra				
	1×5	22,6 ± 0,5	6,4 ≥ 3	4,1	15,1
	Percentage to the control	+22,2		+7,9	+6,3
	1×10	21,3 ± 0,3	5,6 ≥ 3	3,9	14,8
	Percentage to the control	+15,1		+2,6	+4,2

The growth of seedling mass in the dry state was also more actively influenced by the stimulants Crezacin, Ribav-Extra and Ecopin, exceeding the percentage of control, depending on the

concentration of the solution, respectively: by 128.3-176.4 %, 157.5%, and 140.9-151.2 %. Stimulants Zircon and Epin-Extra were less effective (excess to control – 95.3-116.5 % and, respectively: 23.6-48.8 %) (Table 3).

Table 3: The influence of growth stimulants on the formation of the biomass of two-year-old Korean red pine seedlings (*Pinus densiflora* Siebold et Zucc.)

No	Stimulant	The dry weight of seedling in air-dry condition, g					
		trunk	shoots of the 1st order	pine needles	total aboveground part	root system	total weight
1	2	3	4	5	6	7	8
Solution concentration 1ml / 5 l							
1.	Control	0,37	0,01	0,47	0,85	0,42	1,27
1.	Epin-Extra	0,69	0,02	0,61	1,32	0,57	1,89
	Percentage to control	+86,5	+100,0	+29,8	+55,3	+35,7	+48,8
2.	Zircon	0,71	0,02	0,68	1,41	1,07	2,48
	Percentage to control	+91,9	+100,0	+44,7	+65,9	+154,8	+95,3
3.	Crezacin	0,82	0,03	0,79	1,64	1,26	2,90
	Percentage to control	+121,6	+200,0	+68,1	+92,9	+200,0	+128,3
4.	Ribav-Extra	0,94	0,03	0,89	1,86	1,41	3,27
	Percentage to control	+154,1	+200,0	+89,4	+118,8	+235,7	+157,5
5.	Ecopin	0,88	0,02	0,82	1,72	1,34	3,06
	Percentage to control	+137,8	+100,0	+74,5	+102,4	+219,0	+140,9
Solution concentration 1ml / 10 l							
1.	Epin-Extra	0,54	-	0,57	1,11	0,46	1,57
	Percentage to control	+45,9	-	+21,3	+30,6	+9,5	+23,6
2.	Zircon	0,88	0,02	0,71	1,61	1,14	2,75
	Percentage to control	+137,8	+100,0	+51,1	+89,4	+171,4	+116,5
3.	Crezacin	1,34	0,01	0,84	2,19	1,32	3,51
	Percentage to control	+262,2	-	+78,7	+157,6	+214,3	+176,4
4.	Ribav-Extra	1,23	0,01	0,74	1,98	1,29	3,27
	Percentage to control	+232,4	-	+57,4	+132,9	+207,1	+157,5
5.	Ecopin	0,92	0,01	0,87	1,80	1,39	3,19
	Percentage to control	+148,6	-	+85,1	+111,8	+231,0	+151,2

4. CONCLUSION

The experiments showed that in the cultivation of Korean red pine seedlings in the nursery stimulants Crezacin, Ribav-Extra, and Ecopin had a more positive effect.

Root feeding with growth stimulants carried out in the first year retains its positive effect in the second year of seedlings growing in the nursery.

These stimulators increase the growth of seedlings by biometric parameters: height, the diameter of the neck and length of the root lobe, biomass. In the second year of growth in seedlings laying of side shoots buds was marked.

Biennial seedlings in terms of growth meet the requirements of the current OST 56-98-93. They can be used for planting on the forest area and transplanting into the school department of the nursery, for further cultivation of seedlings.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

6. REFERENCES

- Doev, S. K. (2001). Mathematical methods in forestry: textbook. *Ussuriysk: PSAA*, 124 p.
- Galdina, T. E., & Shevchenko, K. V. (2012). Assessment of the influence of biostimulators on the condition and quality of seedlings of European spruce (*Picea abies*) [Electronic resource]. *IV International Student Electron. Scient. Conf. "Student scientific forum"*. Access mode: <https://www.rae.ru/forum2012/13/> 559.
- Golovanov, V. D., Fertikov, V. I., Takhtadjian, A. L., Sokolov, V. E., Skarlato, O. A., Zabrodin, V. A., ... & Geltman, D. V. (1988). Red Book of the RSFSR: Plants.
- Kirienko, M. A., & Goncharova, I. A. (2016). The influence of growth stimulants at different concentrations on ground seed germination and survival of seedlings of the main forest forming species of central Siberia. *Sibirskij Lesnoj Zurnal/Siberian Journal of Forest Science*, (1), 39-45.
- Koryakin, V. N. (Ed). (2010). Handbook for accounting of forest resources of the Far East. responsible compiler and scientific editor. *Khabarovsk: publishing house of FERIF*, 527 p.
- Malevannaya, N. N., & Bykhovskaya, N. V. (2001). Zircon – a new phytopreparation for agriculture, obtained on the basis of unconventional plant raw materials. Chemical and computer modeling. *Butler's reports*, 5, 7.
- Nikitenko, E. A., Gul, L. P., & Korol, L. A. (2005). Study of growth stimulants in the cultivation of planting material of Far Eastern tree species. *Problems of forest protection and multipurpose forest management in the Far East*, 38, 171-175.
- Ostroshenko, V. V., & Ostroshenko, L. Yu. (2010). Influence of stimulants on growth of Korean pine seedlings. *Forestry*, 1, 47-48.
- Ostroshenko, V. V., Ostroshenko, L. Yu., & Ostroshenko, V. Yu. (2015). The use of growth stimulant "Crezacin" in the cultivation of seedlings of the genus "Fir" (*Abies*). *Vestnik of KSAU*, 5, 184-189.
- Ostroshenko, V. V., Poleshchuk, V. A., & Ostroshenko, L. Yu. (2014). Effect of root feeding with stimulant "Epin" on the growth of two-year seedlings of the genus "*Abies* Mill." Theoretical and applied issues of education and science: collection of scientific papers on the materials of the International scientific-practical conference on March 31, 2014: in 13 parts. Part 8. *Tambov: LLC "Consulting company Yukom"*, 120–124.
- Ostroshenko, V. Yu. (2014). The effectiveness of root fertilization with the stimulant "Zircon" in the cultivation of two-year seedlings of Khingan fir (*Abies Nephrolepis* (Trautv.) Maxim. and Manchurian fir (*A. holophylla* Maxim). Problems of sustainable forest management in Siberia and the Far East: materials of All-Russian Conf. with international participation. *Khabarovsk: publishing house of FERIF*, 311-314.
- Pentelkin, S. K. (2001). Application of Agata-25K in forest management. *Forestry*, 2, 41-43.

- Pentelkina, N. V. (2002). Environmentally friendly technologies based on the use of growth stimulants. *Ecology, science, education, upbringing*, 3, 69-71.
- Pentelkina, N. V., & Ostroshenko, L. Yu. (2005). Cultivation of seedlings of coniferous breeds in the North and the Far East with the use of growth stimulants. Actual problems of forestry complex: collection of scientific works of BSITA. *Bryansk*, 10, 125-129.
- Petropavlovsky, B. S. (2004). Forests of Primorsky Krai (Ecological and geographical analysis). *Vladivostok: Dalnauka*, 317 p.
- RMOA. (2017). List of pesticides and agrochemicals approved for use in the Russian Federation. Russian Ministry of Agriculture M., 811 p.
- Red book of Primorsky Krai: Plants*. (2008). Rare and endangered species of plants and fungi. Vladivostok: Orange, 688 p.
- Urusov, V. M., Lobanova, I. I., & Varchenko, L. I. (2004). Coniferous trees and shrubs of the Russian Far East: geography and ecology. *Vladivostok: Dalnauka*, 111 p.
- Urusov, V. M., Lobanova, I. I., & Varchenko, L. I. (2007). Conifers of the Russian Far East – valuable objects of study, protection, breeding, and use. *Vladivostok: Dalnauka*, 440 p.
- Usenko, N. V. (1969). Trees, shrubs, and lianas of the Far East. *Far East Forestry Research Institute, Khabarovsk*.
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