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## EFFECTS OF STIMULANTS ON BIOMETRIC INDICATORS OF GROWTH OF KHINGAN FIR SEEDLINGS (*ABIES NEPHROLEPIS* (TRAUTV.) MAXIM.) IN THE CONDITIONS OF PRIMORSKY KRAI

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### ABSTRACT

The effects of root-feeding stimulators Crezacin, Ribav-Extra, Zircon and Epin-Extra on the growth of seedlings of Khingan fir (*Abies nephrolepis* (Trautv.) Maxim.) as growing planting materials are studied. There was a higher growth activity in seedlings fed with Ribav-Extra growth stimulant at solution concentrations of 1ml/5l and 1ml/10l and Zircon at a concentration of 1ml/10l. Root feeding of seedlings with these stimulants activates their growth in height and biomass, the development of side shoots and the formation of the crown of plants. Three-year-old seedlings can be used for transplanting into the school nursery department, for further cultivation of seedlings and their use in landscape construction. Four-year-old seedlings grown with root-feeding with these stimulants in terms of growth meet the requirements of the current OST 56-98-93 and exceed it in height by 12.3-40.4%, in diameter of the root neck by 6.7-43.3 %.

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## 1. INTRODUCTION

Far Eastern forests are unique, diverse and rich in floristic composition (Petropavlovskii, 2004). Forests of Primorsky Krai are extensive, their total area is 10295.2 thousand hectares; 5341.5 thousand hectares (51.7%) of their area is occupied by coniferous trees. They include pine – 3.8 thousand hectares (0.07%), silver fir – 427.4 thousand hectares (8.0%). Within the Far East, five species of the genus “Fir” (*Abies* Mill.) grow: Khingan (*A. nephrolepis* Maxim.), Manchurian (*A. holophylla* Maxim.), Mayra (*A. mayriana* Miyabe et Kudo), Sakhalin (*A. sachalinensis* Mast.) and Wilson (*A. wilsonii* Miyabe et Kudo). In the forest fund of Primorsky Krai, the most common species is Khingan fir. Fir grows mainly on the mountain slopes, rising to the mountains up to 1200 m, to the zone of loaches. This gives it the importance of mountain protection and water-regulating tree species, contributing to the preservation of ecological balance in the forest fund of the mountain

forests of the Far East (Usenko, 2009).

The wood of the Khingan fir is the least valuable among the coniferous species of the Far East, but in the presence of significant reserves of it, it plays a significant role in forest consumption. It is used as construction and sawing logs, poles. It is good material for box containers, manufacture of plaster shingles. Fir needles are the best raw material for the production of essential oils. It deserves attention in landscaping. We need measures for the conservation and reproduction of fir forests.

The main directions for the conservation of the Far Eastern fir are the protection of forests from forest fires, illegal logging of ripe wood and the intensification of reforestation through the use of growth stimulants, which have proven themselves in experimental work carried out in recent decades in the forestry sector (Galdina & Shevchenko, 2012; Ostroshenko et al., 2014; Ostroshenko, 2014; Ostroshenko et al., 2015; Pentelkina, 2002; Pentelkina & Ostroshenko, 2005).

We have begun studies on the possibility of their use in growing seedlings in forest nurseries.

This work is devoted to the study of the effectiveness of growth stimulants (regulators) of natural (Ribav-Extra, Zircon) and synthetic (Crezacin, Epin-Extra) origin in the cultivation of seedlings of Khingan fir. These growth stimulants have proven themselves positively in agriculture (Vakulenko, 2004; Mukhin, 1979; Nickell, 1994). We have studied the possibility of their application in forestry, in particular – in reforestation (Galdina & Shevchenko, 2012; Ostroshenko et al., 2014; Ostroshenko, 2014; Ostroshenko et al., 2015; Pentelkina, 2002; Pentelkina & Ostroshenko, 2005).

**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 negligible doses activate 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, N2001).

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, and 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” (List of

pesticides and agrochemicals approved for use in the Russian Federation, 2017).

Literature sources reflect three directions for the use of growth stimulants in the nursery: seed treatment before sowing to increase their soil germination, foliar and root fertilization of seedlings (Galdina & Shevchenko, 2012; Ostroshenko et al., 2014; Ostroshenko, 2014; Ostroshenko et al., 2015; Pentelkina, 2002; Pentelkina & Ostroshenko, 2005).

This work studies the effect of root-feeding growth stimulants Crezacin, Ribav-Extra, Zircon, Epin-Extra on one- and two-year-old seedlings of Khingan fir *Abies nephrolepis* (Trautv. Maxim) on their growth and development as in fertilizing period, so and in subsequent years under its ending. Based on this goal, the following tasks were solved:

1. Analysis of forest conditions of the object of works;
2. Harvesting of seeds of Khingan fir and their sowing in the nursery;
3. Carrying out root feeding of one- and two-year-old seedlings with solutions of growth stimulants Crezacin, Ribav-Extra, Zircon, Epin-Extra;
4. Agrotechnical care and the subsequent monitoring of the growth of seedlings height, root system and phytomass during four years;
5. Recording the data into the program Microsoft Excel “STATISTICS” and analysis of the impact 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. Khingan fir seeds collected in autumn 2012 in a wooded area adjacent to the station and in spring 2013 were sown in the nursery beds.

Preparation of the soil consisted of preliminary manual digging of the soil and making beds for the sowing of seeds. The height of the beds is about 20 cm from the soil surface. The location of the seed rows in the beds is transverse. The distance between the centers of the sowing lines is 20 cm, between the variants of the experiments 40 cm. The seeds were soaked in the  $\text{KMnO}_4$  solution before sowing. Depth of sealing – 1.5 cm.

After germination and the beginning of a growing season of the seedlings, then in the middle of the growing seasons of the first and second years of growth (in June), root fertilizing of seedlings with fresh solutions of stimulators was carried out: Crezacin, Ribav-Extra, Zircon, 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. From the third year of seedlings growth, root-feeding was stopped.

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 (stem, 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).

### 3. RESULTS

Analysis of meteorological observations conducted at the research site shows that the weather conditions during the experiments were within the average long-term.

The positive effect of root-feeding with stimulants on the growth of experimental seedlings of Khingan fir was manifested in the first year of growth. We observed an increase in the growth of the root system. So, depending on the concentration of the solution, feeding with the drug Crezacin exceeded the control along the length of the root lobe by 23.7-28.8 %, Ribav-Extra 33.9-37.3%, Zircon 32.2-49.2%, Epin-Extra 28.8-33.9%. Accordingly, the diameter of the root neck - 28.6-42.9%; height 3.6-25.0% (Table 1).

**Table 1:** The impact of root-feeding with stimulants on the growth of annual seedlings of Khingan fir (*Abies nephrolepis* (Trautv.) Maxim.)

№	Growth stimulant / Solution concentration, ml/l	Height, M±m, cm	Materiality of differences	Diameter of root neck, mm	The length of the lobes of the root, cm
1	2	3	4	5	6
1.	Control	2.8 ± 0.1	-	1.4	5.9
2.	Crezacin				
	1×5	2.9 ± 0.1	0.7 ≤ 3	1.9	7.3
	Percentage to the control	+3.6		+35.7	+23.7
	1×10	3.1 ± 0.1	2.1 ≤ 3	2.0	7.6
	Percentage to the control	+10.7		+42.9	+28.8
3.	Ribav-Extra				
	1×5	3.4 ± 0.1	4.3 ≥ 3	2.0	8.1
	Percentage to the control	+21.4		+42.9	+37.3
	1×10	3.2 ± 0.1	2.9 ≤ 3	1.8	7.9
	Percentage to the control	+14.3		+28.6	+33.9
4.	Zircon				
	1×5	2.9 ± 0.1	0.7 ≤ 3	1.8	7.8
	Percentage to the control	+3.6		+28.6	+32.2
	1×10	3.1 ± 0.1	2.1 ≤ 3	1.9	8.8
	Percentage to the control	+10.7		+35.7	+49.2
5.	Epin-Extra				
	1×5	3.5 ± 0.1	5.0 ≥ 3	2.0	7.6
	Percentage to the control	+25.0		+42.9	+28.8
	1×10	3.2 ± 0.1	2.9 ≤ 3	1.9	7.9
	Percentage to the control	+14.3		+35.7	+33.9

Activation of growth of annual seedlings, in combination with the carried-out fertilizing caused further effective growth of biennial seedlings. So, depending on the concentration of the solution, the length of the root lobe exceeded the control in seedlings fed with the stimulant Crezacin by 2.1-5.2 %, Ribav-Extra by 11.5-18.8 %, Zircon 12.5-15.6 % and the stimulant Epin-Extra 2.1-3.1 %; by the diameter of the root neck, respectively, by 5.3-26.3 %; by height-by 7.7-48.7% (Table 2).

**Table 2:** The effect of root fertilizing stimulants on the growth of two-year-old seedlings of Khingan fir (*Abies nephrolepis* (Trautv.) Maxim.)

№	Growth stimulant / Solution concentration, ml/l	Height, M±m, cm	Materiality of differences	Diameter of root neck, mm	The length of the lobes of the root, cm
1	2	3	4	5	6
1.	Control	3.9 ± 0.1	-	1.9	9.6
2.	Crezacin				
	1×5	4.2 ± 0.1	2.1 ≤ 3	2.1	9.8
	Percentage to the control	+7.7		+10.5	+2.1
	1×10	4.7 ± 0.1	5.7 ≥ 3	2.3	10.1
	Percentage to the control	+20.5		+21.1	+5.2
3.	Ribav-Extra				
	1×5	5.2 ± 0.1	9.3 ≥ 3	2.4	11.4
	Percentage to the control	+33.3		+26.3	+18.8
	1×10	4.6 ± 0.1	5.0 ≥ 3	2.2	10.7
	Percentage to the control	+17.9		+15.8	+11.5
4.	Zircon				
	1×5	4.7 ± 0.1	5.7 ≥ 3	2.0	10.8
	Percentage to the control	+20.5		+5.3	+12.5
	1×10	5.2 ± 0.1	9.3 ≥ 3	2.3	11.1
	Percentage to the control	+33.3		+21.1	+15.6
5.	Epin-Extra				
	1×5	5.8 ± 0.1	13.6 ≥ 3	2.1	9.9
	Percentage to the control	+48.7		+10.5	+3.1
	1×10	4.6 ± 0.1	5.0 ≥ 3	2.0	9.8
	Percentage to the control	+17.9		+5.3	+2.1

Feeding of biennial seedlings with these growth stimulants with a solution concentration of 1ml/10 l exceeded the control in height by 17.9-33.3%, in the diameter of the root neck by 5.3-21.1%, in the length of the root lobe – by 2.1-15.6%; at a lower concentration by 7.7-48.7%, 5.3-26.3%, and 2.1-18.8%. The materiality of the differences is in the range of 5.0-13.6.

At the end of the second year of growth in experienced seedlings, the beginning of crown formation was marked. Thus, when root feeding with a Zircon solution concentration of 1ml/10l in 44.0 % of seedlings, the laying of buds of side shoots were noted; at a concentration of 1ml/5l in 18.0 %.

In three-year-old seedlings from the laid side buds, the development of side shoots began. Since the third year of growth, root fertilization of seedlings was not carried out, but the positive effect of stimulants on their growth, observed in the first two years, continued.

The growth activity of seedlings was observed in the fourth year of their growth (Table 3). Depending on the drug and the solution concentration in four-year seedlings, the average growth rates exceeded the control: in height, when fed with the stimulant Crezacin by 12.3-24.0%, Ribav-Extra by 26.0-40.4 %, Zircon by 24.7-28.1 %, Epin-Extra by 15.1-21.9 %; in the diameter of the root neck, respectively by 6.7-43.3 %; in the length of the root lobe by 1.4-46.6 %.

The tested growth stimulators had a positive effect on the biomass growth of four-year seedlings of Khingan fir. Thus, depending on the concentration of the solution, the percentage of excess to control when root fertilizing of seedlings with the stimulant Crezacin was 69.5-89.7%, Ribav-Extra 235.6-268.2%, Zircon 198.3-286.7% and Epin-Extra 76.0-85.8% (Table 4, Figure 1).



**Table 3.** The effect of root fertilizing stimulants on the growth of four-year-old seedlings of Khingan fir (*Abies nephrolepis* (Trautv.) Maxim.)

№	Growth stimulant / Solution concentration, ml/l	Height, M±m, cm	Materiality of differences	Diameter of root neck, mm	The length of the lobes of the root, cm
1	2	3	4	5	6
1.	Control	14.6 ± 0.3	-	3.0	14.8
2.	Crezacin				
	1×5	16.4 ± 0.2	5.0 ≥ 3	3.2	15.9
	Percentage to the control	+12.3		+6.7	+7.4
	1×10	18.1 ± 0.2	9.7 ≥ 3	3.7	16.1
	Percentage to the control	+24.0		+23.3	+8.8
3.	Ribav-Extra				
	1×5	20.5 ± 0.2	16.4 ≥ 3	4.3	21.7
	Percentage to the control	+40.4		+43.3	+46.6
	1×10	18.4 ± 0.2	10.6 ≥ 3	3.8	18.7
	Percentage to the control	+26.0		+26.7	+26.4
4.	Zircon				
	1×5	18.2 ± 0.2	10.0 ≥ 3	3.2	17.9
	Percentage to the control	+24.7		+6.7	+20.9
	1×10	18.7 ± 0.4	8.2 ≥ 3	3.3	15.0
	Percentage to the control	+28.1		+10.0	+1.4
5.	Epin-Extra				
	1×5	17.8 ± 0.1	10.0 ≥ 3	3.4	17.5
	Percentage to the control	+21.9		+13.3	+18.2
	1×10	16.8 ± 0.4	4.4 ≥ 3	3.5	15.7
	Percentage to the control	+15.1		+16.7	+6.1

**Table 4:** Influence of growth stimulants on the formation of the biomass of four-year seedlings of Khingan fir (*Abies nephrolepis* (Trautv.) Maxim.)

No	Stimulant	The number of the first order twigs, pieces	Dry weight of seedling in air-dry condition, g					
			Trunk	Twigs	Fir needle	Total aboveground part	Root system	Total weight
1	2	3	4	5	6	7	8	9
Solution concentration 1ml / 5 l								
1.	Control	4	0.45	0.15	1.17	1.77	0.56	2.33
1.	Epin-Extra	5	0.87	0.42	1.83	3.12	1.21	4.33
	Percentage to the control	+25.0	+93.3	+180.0	+56.4	+76.3	+116.1	+85.8
2.	Zircon	4	1.68	0.73	3.21	5.62	1.39	6.95
	Percentage to the control	-	+273.3	+386.7	+174.4	+217.5	+148.2	+198.3
3.	Crezacin	6	1.16	0.28	1.44	2.88	1.07	3.95
	Percentage to the control	+50.0	+157.8	+86.7	+23.1	+62.7	+91.1	+69.5
4.	Ribav-Extra	7	1.78	0.96	3.32	6.06	1.76	7.82
	Percentage to the control	+75.0	+295.6	+540.0	+183.8	+242.4	+214.3	+235.6
Solution concentration 1ml / 10l								
1.	Epin-Extra	5	0.82	0.35	1.79	2.96	1.14	4.10
	Percentage to the control	+25.0	+82.2	+133.3	+53.0	+67.2	+103.6	+76.0
2.	Zircon	6	1.74	0.96	3.86	6.56	2.45	9.01
	Percentage to the control	+50.0	+286.7	+540.0	+229.9	+270.6	+337.5	+286.7
3.	Crezacin	7	0.90	0.39	1.94	3.23	1.19	4.42
	Percentage to the control	+75.0	+100.0	+160.0	+65.8	+82.5	+112.5	+89.7
4.	Ribav-Extra	7	1.61	0.89	3.97	64.7	2.11	8.58
	Percentage to the control	+75.0	+257.8	+493.3	+239.3	+265.5	+276.8	+268.2

## 4. CONCLUSION

The first experiments allow us to conclude about the possibility of applying growth stimulants in the cultivation of planting material of Khingan fir in forest nurseries. Higher growth activity was observed in seedlings fed with growth stimulant Ribav-Extra at concentrations of solution 1ml/5L and 1ml/10l and Zircon – at concentrations of 1ml /10l.

Root feeding of seedlings with these stimulants activates their growth in height and biomass, the development of side shoots and the formation of the crown of plants.

Three-year seedlings can be used for transplanting into the school nursery department, for further growing seedlings and their use in landscape construction.

Four-year seedlings grown with root fertilization with these stimulants in terms of growth meet the requirements of the current OST 56-98-93 (1993), and exceed it in height by 12.3-40.4%; in diameter of the root neck 6.7-43.3%.

Carried out in the first two years of seedlings growth root fertilization with growth stimulants retains its effective impact in the next two years. It is necessary to find out to what age of plants it is possible to prolong the action of growth stimulants.

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