

# Physiological response to excessive doses of indolyl-3-butyric acid and its potassium salt in stimulating the rooting of semi-woody cuttings of coniferous crops

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**Abstract.** The study of root formation stimulation in cuttings of coniferous crops is often accompanied by a decrease in survival rate with an increase in the content of indolyl-3-butyric acid or its potassium salt in the regulator above a certain value. In contrast to cases of hormone deficiency in the stimulant, when cuttings remain alive, but root formation does not occur or a callus is formed, with excessive concentrations, the death of cuttings is observed. Under the conditions of film cutting tunnels equipped with substrate heating, observations were made on the condition of cuttings of coniferous crops in variants with an increasing concentration of exogenous auxin, leading to a decrease in survival rate. It is shown that in such variants, the appearance of cuttings with a "rotten base" is observed, which are a transitional form between healthy well-rooted cuttings and completely dead ones.

## 1 Introduction

In the vegetative propagation of coniferous crops by rooting of cuttings, treatments with stimulants containing auxins are widely used. The most common use of indolyl-3-butyric acid (IBA) and its potassium salt (IBA-P), which are used in the form of aqueous, alcoholic solutions or contained in growth powders [1]. Soaking the bases of cuttings in aqueous solutions of IBA for 18-24 hours is the most stable way to stimulate root formation, all other things being equal, which explains its widespread use in research work. The effective concentration of the exogenous hormonal regulator depends on the culture, type, variety, and physiological factors of the cuttings and usually ranges from 30 to 150-200 mg/l of IBA, while it is noted that an aqueous solution of IBA with a concentration of 100 mg/l is often the most optimal [2,3].

At the same time, it is known that excessive doses of auxins have a phytotoxic effect. Possible physiological causes of this phenomenon may be the suppression of plant protective reactions [4] or auxin stimulation of the aging hormone ethylene release and the corresponding physiological reactions of tissue damage leading to the death of cuttings [5]. Apparently, it is the phenomenon of phytotoxicity that explains the phenomenon observed in

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studies of a decrease in the survival rate of cuttings with an increase in the concentration of auxin in the rooting stimulant. For example, when soaking cuttings in an aqueous IMA solution with a concentration change from 50 mg/l to 100 mg/l, rooting was significantly reduced in coniferous plants such as Chinese juniper "Blue Point" (*Juniperus chinensis* L. "Blue Point"), Rocky Mountain juniper varieties "Skyrocket" and "Blue Arrow" (*Juniperus scopulorum* Sarg. "Skyrocket", "Blue Arrow"), red juniper "Grey Owl" (*Juniperus virginiana* L. "Grey Owl") [1], blue spruce "Alberta Globe", "Blue Variegated", "Conica", "Sander's Blue" (*Picea pungens* Engelm. "Alberta Globe", "Blue Variegated", "Conica", "Sander's Blue") [1], opposite cross-pair microbiota (*Microbiota decussata* Kom.) [3]. A decrease in survival rate was observed with short-term immersion in an alcoholic solution of IMA above the level of 14 mM (2.8 g/l) for cuttings of Fraser fir (*Abies fraseri* Poir.) [6]. When using growth powders with different IMA content, the same trend is observed. Thus, for rooting of cuttings of white cedar "Smaragd" (*Thuja occidentalis* "Smaragd") when using growth powders with a IMA content of 0.1-0.9%, the optimal concentrations were 0.3-0.6%, whereas higher concentrations reduced survival [7].

The use of phytotoxic doses of a stimulant in scientific papers can lead to contradictory results. For example, in studies where a stimulant containing IMA in a phytotoxic dose is used without preliminary reconnaissance, especially for easily rooted crops, the percentage of engrafted cuttings is lower than the control variant, where the stimulant is not used. Thus, when rooting the white cedar "Danica", 75.1% of cuttings took root in the control, and when using Kornevin powder with IMA content of 5 g/kg, this indicator decreased to 61.3% [8]. Since auxins are the main hormones that stimulate rooting, they are used as control options for testing other physiologically active substances in a similar quality. In this case, an unsuccessful selection of auxin concentrations, at which phytotoxicity and a decrease in the percentage of rooting may occur, may lead to incorrect conclusions about the effectiveness of the compared preparations [9, 10, 11].

In connection with the above, processing the results of the experiments, in addition to survival, it would be desirable to have additional rooting indicators that would allow to more fully characterize the effectiveness of the auxin series stimulant. In this way, in previous works, we proposed to pay more attention to such a characteristic as callus formation, when the presence of a large percentage of rooted and non-rooted cuttings with callus in the variants may indicate a lack of auxin in an exogenous stimulator of root formation [12]. Additionally, when examining the rooted cuttings of easily rooted coniferous species in our production using a root formation stimulator, we drew attention to the presence of a "rotten base" phenomenon. The "rotten base" of a cut is the inanimate basal part of an ingrained cut from the base to the first lower living root (Figure 1) [13]. To determine the relationship between the appearance of a "rotten base" and an auxin rooting stimulant, we included this indicator in the calculation of the results of current experiments with the testing of stimulants with IBA and IBA-P in increasing concentrations.

## 2 Materials and Methods

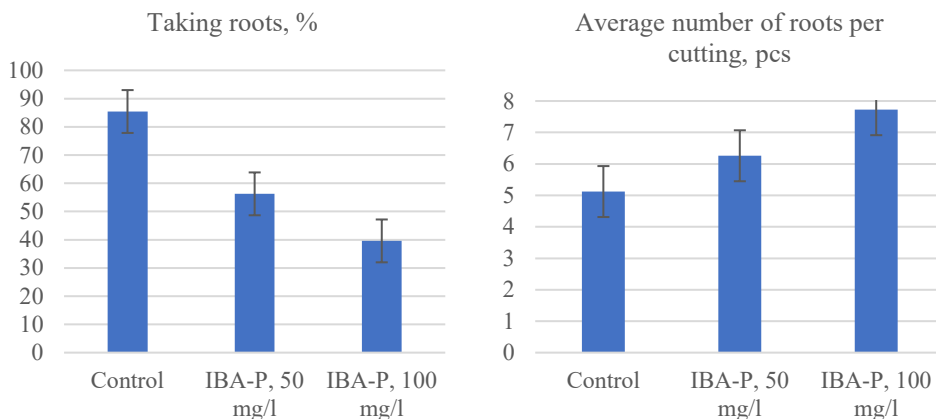
The research was conducted in 2021-2022 on the basis of the production nursery of ornamental plants "Vashutino" in the department located in the city of Zubtsov, Tver region. Semi-woody cuttings of coniferous crops were used in the research - *Thuja occidentalis* "Smaragd", Rocky Mountain juniper "Skyrocket", Pfitzer juniper "Pfitzeriana Glauca" (*Juniperus x pfitzeriana* "Pfitzeriana Glauca") savin juniper (*Juniperus sabina* L.). Rooting of cuttings was carried out in film cutting tunnels with a height of 0.6-0.9 m, equipped with low-pressure fog and underfloor heating. The cuttings were placed inside a film hangar greenhouse equipped with air heating and fine-drop air cooling [13]. Additionally, the greenhouse under the roof is equipped with a white spunbond cover with a density of 60 g/m.

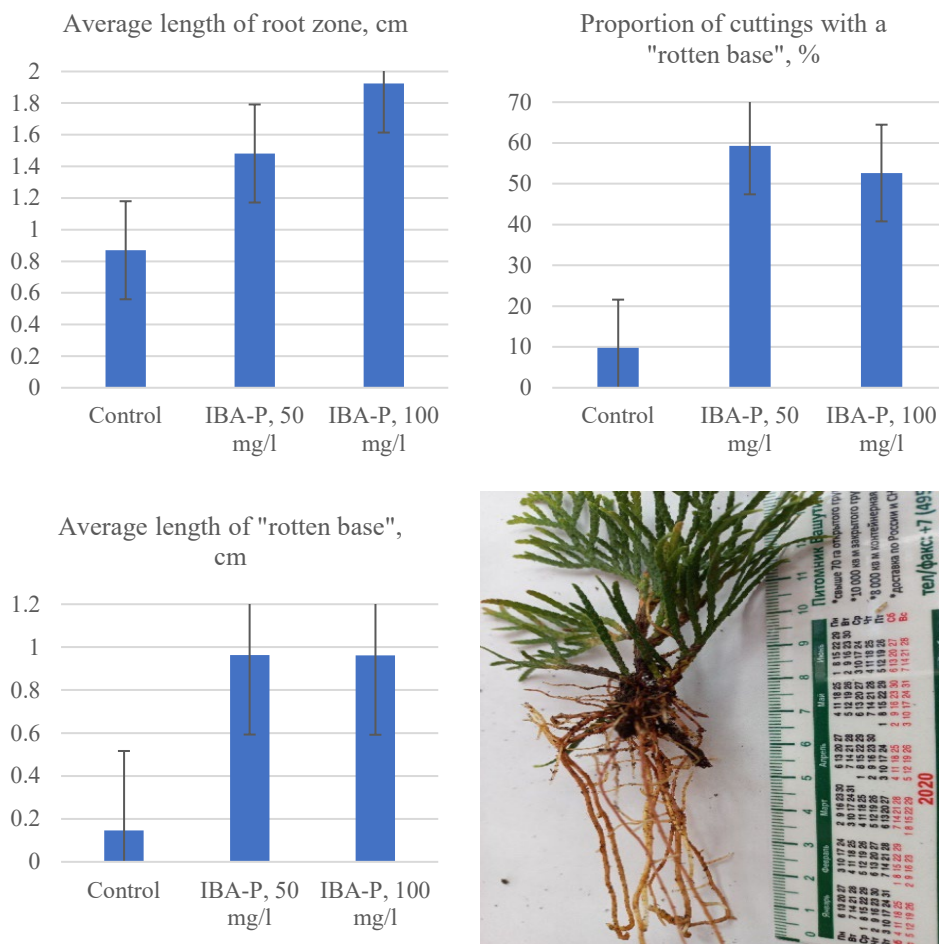
The average mode of switching on the fog-forming unit on sunny days was 5 seconds per 30 minutes, on cloudy days the cuttings were sprayed once a day. To stimulate rooting, indolyl-3-butyric acid aqueous solutions of potassium salt (IBA-P, China) were used in which the bases of cuttings were soaked 3-4 cm for 16-18 hours and growth powders - Chrysotop green 0.25% (IBA content 2.5 g/kg, Rhizopon production, the Netherlands), Kornevin (IBA content 5 g/kg, production of Agrosynthesis, Russia), Rhizopon AA 1% (IBA content 10 g/kg, manufactured by Rhizopon, the Netherlands). In part of the experiments, the concentration gradient of the active substance was obtained by diluting the Kornevin powder with talc in the following ratios 1:1, 1:2, 1:3, accordingly. According to the technological scheme, the cuttings were planted in cassettes, immediately before planting they were dipped completely into a solution of fundazole 5 g/10 liters. In experiments with powders, the bases of wet cuttings, according to the method [14], were powdered with stimulants and planted in cassettes by sticking the cuttings into the substrate to a depth of 3-4 cm. Cuttings rooted without a stimulant served as a control. In those experiments where the bases of the cuttings were soaked in stimulant solutions, the control cuttings were soaked in distilled water. As a substrate for rooting, a mixture of slightly decomposed peat pH 4.8-5.3 was used with a preliminary introduction of Trichocine in a dose of 30 g/cubic meter in 4-5 days and agroperlite of 1-5 mm fraction in a ratio of 2 parts of peat to 1 part of perlite. The experimental cassettes were placed in cuttings in blocks among the production cassettes. The results of the experiments were processed according to the variance analysis [15].

### 3 Results and Discussions

*Thuja occidentalis* "Smaragd" refers to easily rooted varieties and takes root well without stimulants. Therefore, in the experiment of soaking the bases of cuttings in aqueous solutions of IBA-P 50 mg/l and 100 mg/l, the stimulant immediately showed its phytotoxic effect, reducing survival by 29.16% and 41.83%, respectively, relative to the control. At the same time, a significant number of cuttings with a "rotten base" appeared, the proportion of which in the variants was 59.3% and 52.6%, respectively, and the average length of the "rotten base" was 0.96 cm in both variants (Figure 1). Despite its phytotoxicity, the stimulant improved the quality of the root system: the number of roots in the variants increased by 1.22 and 1.51 times, respectively, and the length of the root zone increased by 1.7 and 2.21 times.

Figure 1. Indicators of rooting of semi-woody cuttings of *Thuja occidentalis* "Smaragd" when stimulating rooting with aqueous solutions of IBA-P. The age of the mother plants is 3-4 years. Planting for rooting on 01/27/2022, counting the results – 08/04/2022. Photo of a cutting base with a "rotten base".

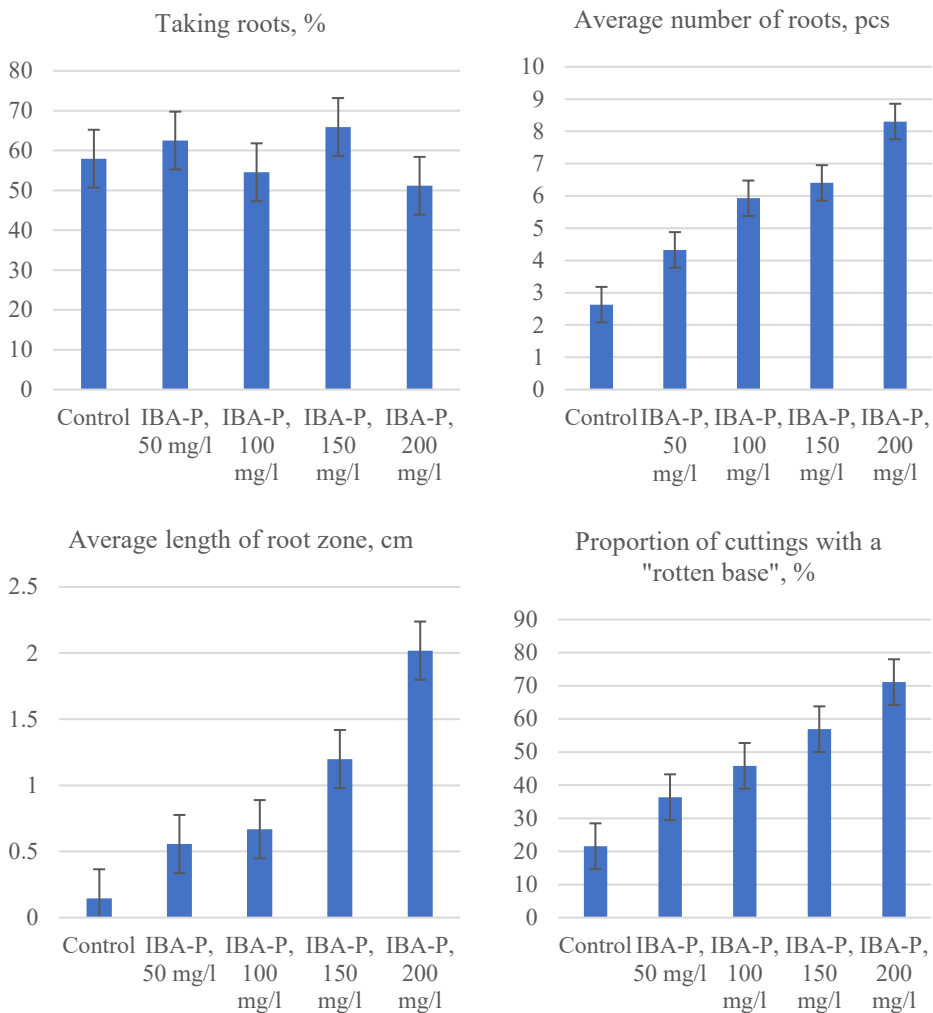


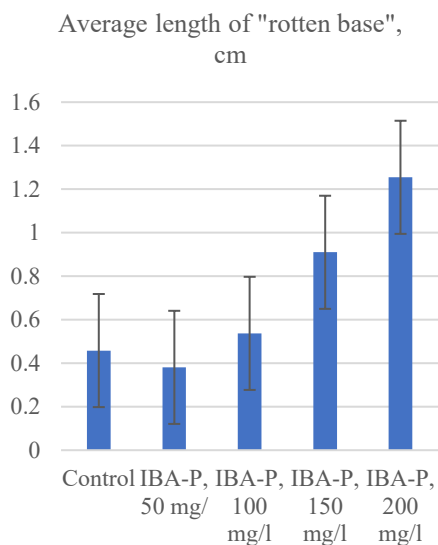


**Fig. 1.** Indicators of rooting of semi-woody cuttings of *Thuja occidentalis* "Smaragd" when stimulating rooting with aqueous solutions of IBA-P. The age of the mother plants is 3-4 years. Planting for rooting on 01/27/2022, counting the results – 08/04/2022. Photo of a cutting with a "rotten base".

When rooting Rocky Mountain juniper "Skyrocket", soaking the bases of cuttings in stimulating aqueous solutions of IBA-P with concentrations from 50 mg/l to 200 mg/l was used (Figure 2). The survival rate in all variants did not differ significantly from the control (LSD 0.5=14.52%) and varied between 51.13-65.9%. In the control variant, the formation of a "classical" root system for rock junipers was observed – 2-3 roots of the first order at the base of the cuttings [13]. The quality of the root system improved with an increase in the concentration of IBA-P and in the variant with IBA-P 200 mg/l, the root system had an average of 8.3 roots, the root zone extended to a length of 2.02 cm. Simultaneously with an increase in the IBA-P in the solution, the number of cuttings that had a "rotten base" increased, and the average length of this indicator increased. In the control variant, the number of cuttings with a "rotten base" was 21.57% and, accordingly, was caused by factors unrelated to the stimulant, but already in the variant with IBA-P 50 mg/l, the proportion of such cuttings increases by 1.69 times to 36.35% and this increase is statistically significant (LSD 0.5 =13.8%). In the variant with IBA-P 200 mg/l, the proportion of cuttings with a "rotten base" increases by 3.3 times compared with the control, and its length by 2.74 times.

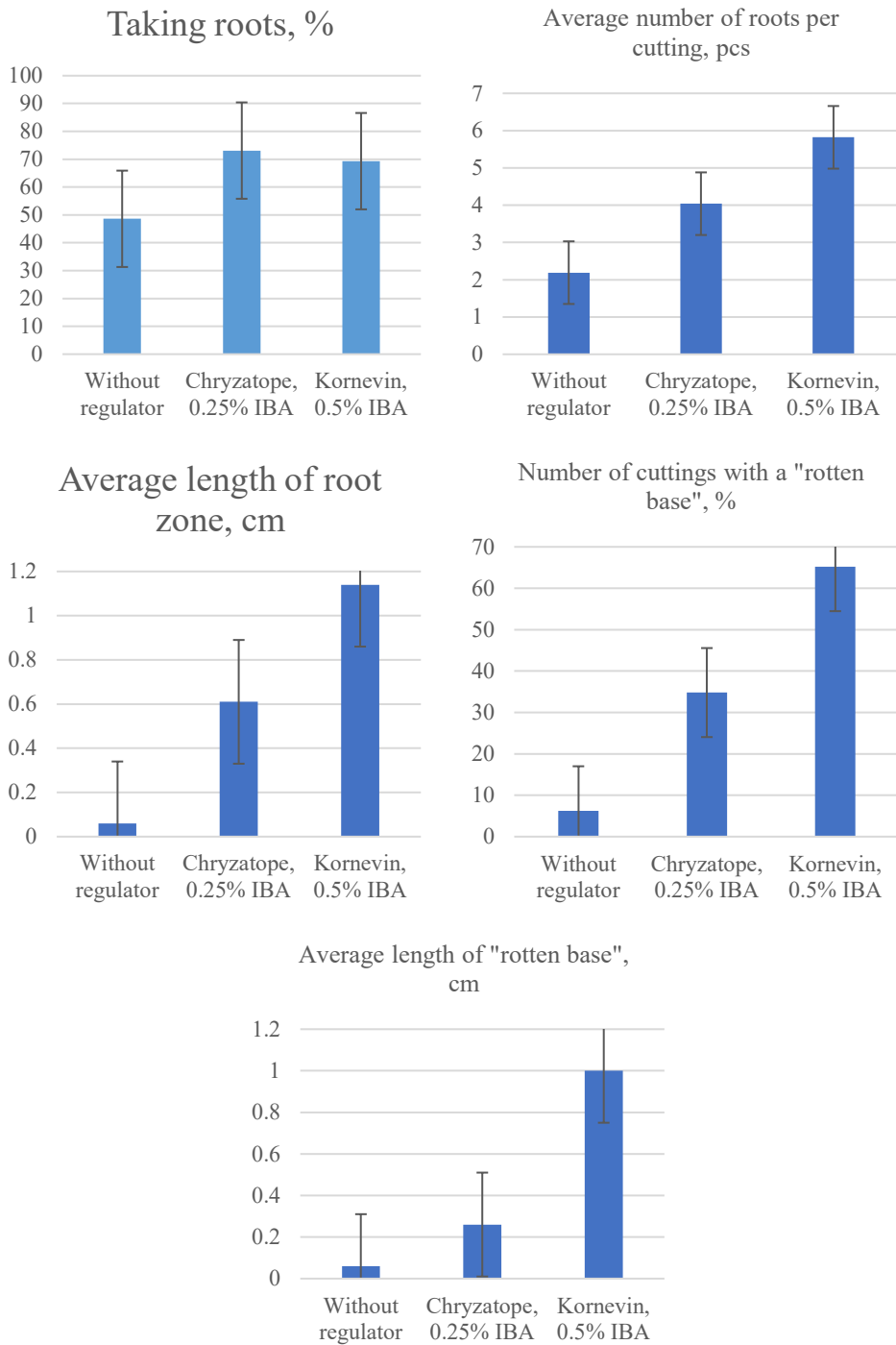
Thus, with an increase in the concentration in solutions of IBA-P, signs of improvement in the quality of the root system and signs of phytotoxicity are simultaneously observed. The best survival rate of cuttings is achieved in the variant with a IBA-P 150 mg/l, and in the variant with 200 mg/l rooting significantly decreases by 14.77%, which may indicate an excess of phytotoxicity over the stimulation of rooting.





**Fig. 2.** The change in rooting indices of semi-woody cuttings of Rocky Mountain juniper "Skyrocket" with an increase in the content of IBA-P in stimulating solutions. The age of the mother plants is 10 years. Planting on 01/27/2022, counting the results – 10/10/2022. Photos of cuttings with a "rotten base" of different lengths.

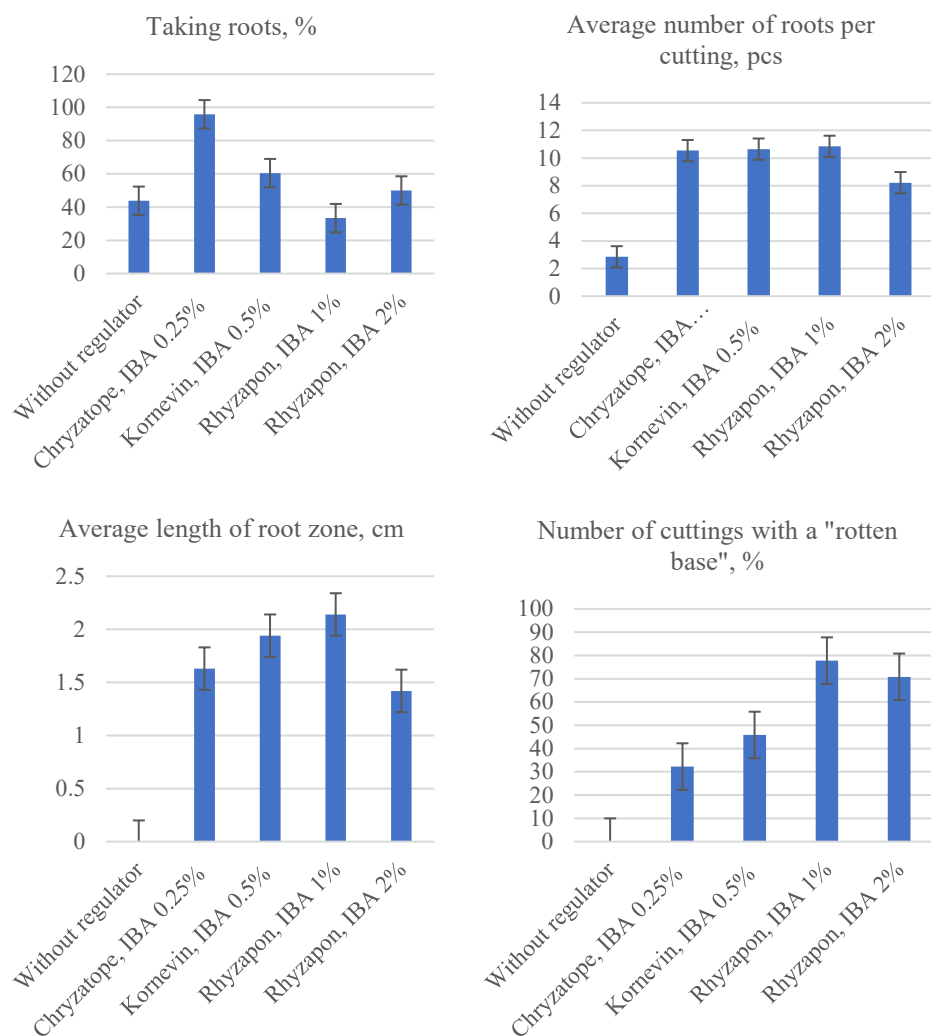
We also observed manifestations of a "rotten base" in cuttings in experiments using growth powders with different IBA concentrations as rooting stimulators. Semi-woody cuttings of Rocky Mountain juniper "Skyrocket" were rooted using Chrysatope 0.25% growth powders and Kornevin. In both variants, there was an increase in rooting compared to the control by 24.5% and 20.7%, respectively. With an increase in the concentration of IBA powder, the rooting result did not increase, but signs of phytotoxicity increased. Thus, the number of cuttings with a "rotten base", which turned out to be a minimum amount of - 6.25% in the control, increased 5.6 times in the variant using 0.25% Chrysatope, and 10.4 times in the variant with Kornevin, the average length of the "rotten base" was 0.26 cm and 1.0 cm, respectively. In absolute terms, the size of this indicator in the control was in the range of 0-1 cm and was most likely determined by random factors. In the variant with Chrysatope 0.25%, the length of the "rotten base" varied already within the range of 0-2 cm, and in the variant with Kornevin within 0-3 cm. Statistical data processing [15] showed a significant influence of the studied factor on these indicators.



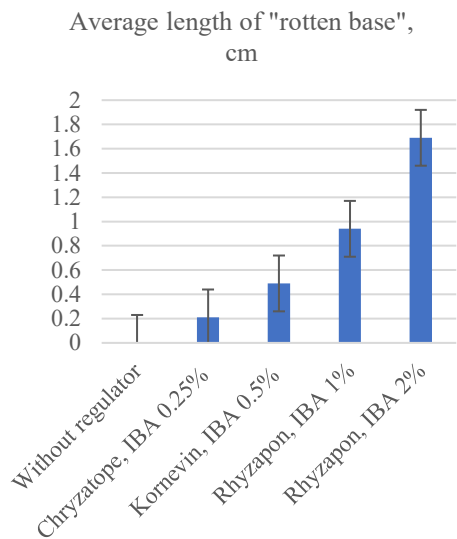
**Fig. 3.** Indicators of rooting of semi-woody cuttings of Rocky Mountain juniper "Skyrocket". The age of the mother plants is 9 years. Planting for rooting on 01/16/2021, counting the results – 09/29/2021.

The stimulating effect of growth powders with different IBA concentrations was studied when rooting semi-woody cuttings of Pfitzer juniper "Pfitzeriana Glauca". The experiment

was conducted, among other things to determine the natural rooting ability of this variety [16]. Cuttings in the control without treatments took root by 43.8%, which made it possible to classify this variety as easily rooted. Therefore, it is not by chance that the best options are the survival rate of cuttings of 95.85%, Chryzatope green 0.25% with the lowest IBA concentration of the used stimulants turned out to be. An increase in the IBA content in the powder above 0.25% led to signs of phytotoxicity. Thus, in the variants using Kornevin, Rhizopon 1%, and Rhizopon 2%, the survival rate of cuttings decreased by 35.4%, 62.5%, 45.85%, respectively. In all variants of the experiment, except for the control, there were cuttings with a "rotten base". In the variant with Chryzatope green 0.25%, there were 32.26% with an average length of this zone of 0.21 cm, which may indicate some phytotoxicity of this low IBA concentration. With an increase in IBA concentration in other variants, the proportion of cuttings with a "rotten base" increased by 13.57%, 45.52%, and 38.57%, and its length by 0.28 cm, 0.73, cm and 1.48 cm, respectively.

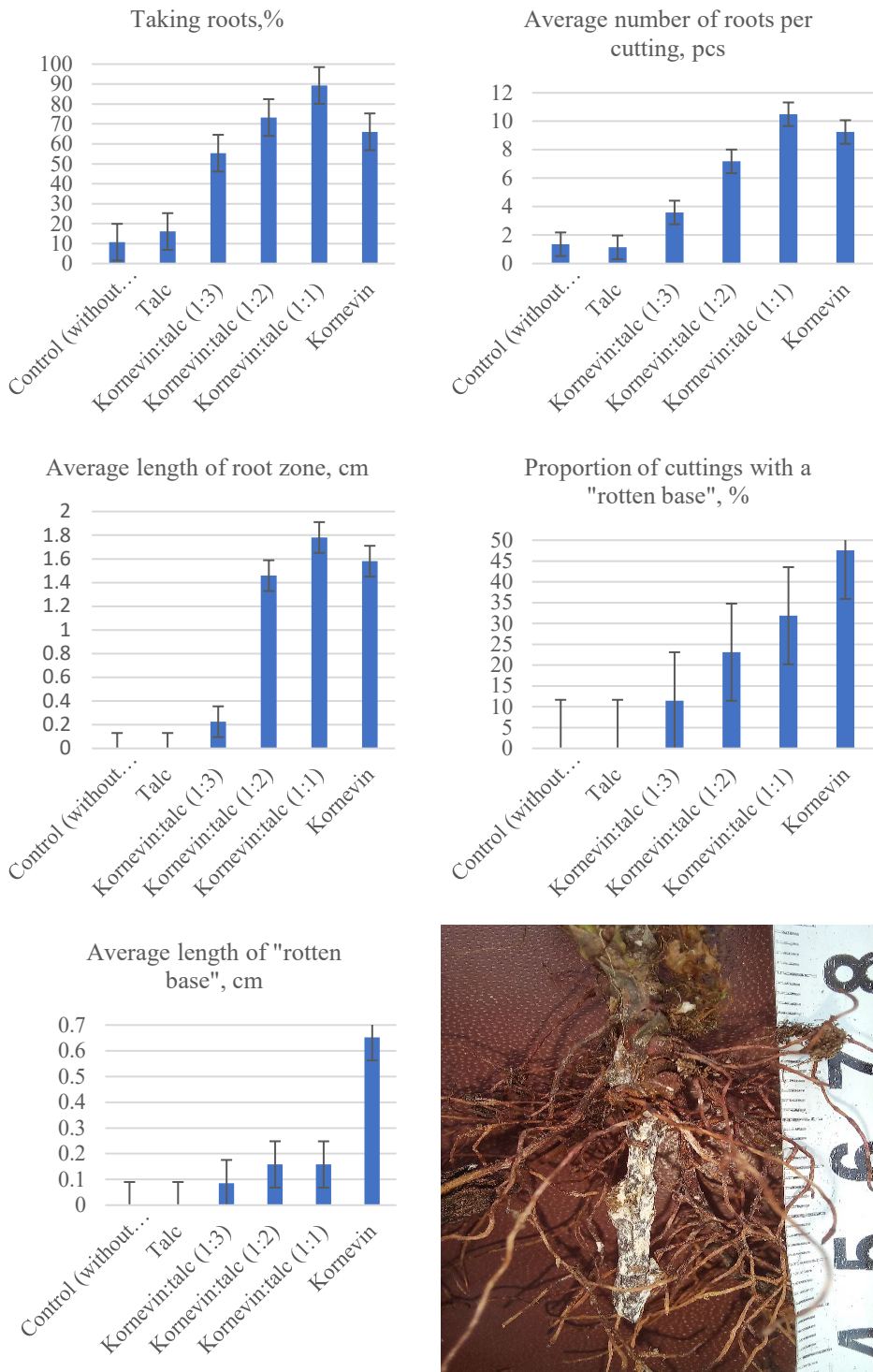






**Fig. 4.** Indicators of rooting of semi-woody cuttings of Pfitzer juniper "Pfitzeriana Glauca". The age of the mother plants is 7 years. Planting for rooting on 03/22/2021, counting the results – 11/10/2021. Photo of a cutting with a "rotten base".

When rooting semi-woody cuttings of savin juniper, Kornevin powder was used as a root formation stimulant (Figure 5). To ensure a concentration gradient of IBA from 0 to 5 g/l, the following weight mixtures of talc and Kornevin were used: 1 to 0, 3 to 1, 2 to 1, 1k1 and 0 to 1, which corresponds to the following IBA concentrations: 0 g/kg, 1.25 g/kg, 1.67 g/kg, 2.5 g/kg, and 5g/kg. Cuttings were harvested from plants eight years old. Despite the fact that this crop is also considered to be easily rooted, cuttings in the control variant took root by only 10.71%. Perhaps this is due to the age of the mother plants, which is large enough for this culture. With an increase in the IBA concentration in the powder from 0 to 2.5 g/l, all rooting indicators increased – the survival rate reached 89.29%, the number of roots per cuttings was 10.49, which is 8.34 and 7.71 times more than the control, respectively, the length of the root zone increased from 0 to 1.78 cm. Phytotoxicity indicators behaved similarly – the proportion of cuttings with a "rotten base" increased from 0 to 31.86%, the average length of the "rotten base" zone increased to 0.16 cm, on individual cuttings it reached 2 cm. Despite the significant proportion of cuttings with a "rotten base", powder with IBA content of 2.5 g/kg (Kornevin: talcum powder 1:1) was the best option for survival. With a further increase in IBA concentration to 5 g/kg (Kornevin without dilution), the survival rate of cuttings decreased sharply by 23.19%, the quality of the root system did not change significantly, but, as expected, the growth of phytotoxicity signs continued – the number of cuttings with a "rotten base" increased by another 15.71% reaching 47.57%, the length of this zone sharply increased to 0.65 cm, the absolute length increased to 3 cm.



**Fig. 5.** The change in rooting indices of semi-woody cuttings of savin juniper with an increase in IBA content in stimulating powders. The age of the mother plants is 8 years. Planting for rooting on 04/30/2022, counting the results – 11/04/2022. Photo of a cutting with a "rotten base".

## 4 Conclusions

In the practice of propagation of coniferous crops by cuttings, the phenomenon of "rotten base" is observed quite often. The reasons for this are several interrelated factors: soaking of cuttings with excessive moisture, high substrate density, increased salt content in the substrate, deep planting of cuttings, as well as tissue damage by fungal diseases. Our research shows that when selecting the optimal substrate and using healthy queen cells, the "rotten base" of cuttings is a consequence of the high content of the auxin hormone in the rooting stimulant. Up to a certain IBA concentration or its potassium salt in solutions and powders, the sum of positive stimulating properties and phytotoxic qualities create a generally positive rooting effect, expressed in an increase in the percentage of engrafted cuttings. With a further increase in concentration, despite the preservation of good quality of the root system, a decrease in survival occurs, accompanied by an increase in the proportion of dead cuttings and cuttings with a "rotten base", which are a transitional form from healthy well-rooted cuttings and completely died as a result of hormonal phytotoxicity of IBA and IBA-P in the regulator.

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