

Influence of clonex and indole-3-butyric acid on the rhizogenesis of *Picea pungens* f. *glauca* cuttings

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Abstract. The study aimed to provide a quantitative assessment of the effects of different concentrations of indole-3-butyric acid (IBA) and gel-based formulations on the intensity of rhizogenesis in semi-hardwood cuttings of *Picea pungens* f. *glauca*, with the objective of identifying the most effective treatment options. Over a three-year period (2023-2025), the efficacy of Clonex Green, Clonex Purple, and Clonex Red, as well as aqueous IBA solutions at concentrations of 1,000, 3,000, and 5,000 mg L⁻¹, was evaluated in the propagation of semi-hardwood cuttings of *Picea pungens* f. *glauca*. Cuttings 8-12 cm in length were collected from stock plants up to 10 years of age and established in a substrate composed of a 1:1 mixture of river sand and highmoor peat. The results demonstrated that the application of Clonex formulations and IBA significantly increased the rooting percentage compared with the control treatment, where the mean value was only 5.6%. The highest efficacy was achieved with the gel formulation Clonex Purple, which resulted in a rooting percentage of 37.8%. High effectiveness (28.9%) was also observed with Clonex Green and with the aqueous IBA solution at a concentration of 1,000 mg L⁻¹. It was found that a further increase in the concentration of pure IBA to 3000 mg L⁻¹ led to a decrease in rooting to 24.4%, while the application of 5,000 mg L⁻¹ caused a pronounced inhibition of rhizogenesis (11.1%), indicating a phytotoxic effect. Gel-based stimulants (Clonex) were shown to outperform aqueous solutions, which can be attributed to prolonged contact of the active substance with the base of the cutting, its gradual release, and protection against leaching from the substrate. Statistical analysis using analysis of variance confirmed the significance of the observed differences (LSD_{0.05} = 1.71-1.83). The findings provide a basis for recommending optimal concentrations of growth stimulants to improve the production of high-quality planting material of ornamental coniferous species

Keywords: vegetative propagation; plant rooting; auxins; adventitious roots; rooting stimulators

INTRODUCTION

Efficient propagation of ornamental coniferous species remains a key objective in modern ornamental horticulture and forestry. Particular attention is given to blue spruce (*Picea pungens* f. *glauca*), which is widely used in urban landscaping due to its high ornamental value and tolerance to adverse environmental conditions. However, generative propagation does not ensure the preservation

of its morphological traits, thereby necessitating the use of vegetative methods. Among these, stem cuttings represent one of the most promising approaches for producing uniform planting material, although rooting efficiency in conifers remains relatively low, which highlights the need to develop effective strategies for stimulating rhizogenesis and improving rooting technologies.

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A critical role in the propagation of woody plants by cuttings is played by phytohormones, particularly auxins, as demonstrated in study analysing the physiological and molecular mechanisms of adventitious root formation, where S. Liu *et al.* (2025) showed that indole-3-butyric acid (IBA) promotes rhizogenesis by activating cell division in vascular tissues and initiating the formation of root primordia. This mechanism contributes to increased rooting percentages and improved root system morphology, confirming the relevance of IBA application in vegetative propagation systems. The stimulatory effect of IBA on adventitious root formation has also been confirmed in experimental study on woody species, where M.A. Ibrahim *et al.* (2025), investigating rhizogenesis in *Zanthoxylum beecheyanum*, demonstrated that exogenous auxin application enhances cell division in the cambial zone and initiates root primordia development, resulting in higher rooting rates and improved root system architecture compared with untreated controls. These findings further substantiated the pivotal role of auxins in regulating rhizogenic processes.

A synthesis of contemporary research indicated that growth regulators substantially improve the efficiency of vegetative propagation in woody plants, with S.J. Trueman *et al.* (2021) demonstrating, through an extensive analysis of auxin-mediated adventitious root induction, that exogenous application of IBA increases rooting success, promotes the formation of a greater number of roots, and enhances their subsequent development, while its effectiveness is strongly dependent on concentration, physiological status of donor plants, cutting type, and environmental conditions. This highlighted the complex regulatory role of IBA in rhizogenesis. Further evidence of the effectiveness of IBA has been reported in studies on *Ficus benjamina*, where Y.P. Zala & M.M. Masu (2025) established that concentrations of 1,000-2,000 mg L⁻¹ significantly stimulate root formation, increasing root number, length, and total biomass, with the highest morphological parameters observed at 2,000 mg L⁻¹, indicating a pronounced dose-dependent stimulatory effect.

In contemporary research, considerable attention is devoted to the use of commercial auxin-based formulations to enhance the rooting of woody plant cuttings. Among these, *Clonex Rooting Gel*, containing indole-3-butyric acid at a concentration of 0.31%, has been widely investigated. In a study on the clonal propagation of coniferous species, it was demonstrated by J.B. Silva Filho *et al.* (2026), working with *Pinus lambertiana* and *Pinus ponderosa*, that the application of Clonex led to a marked increase in rooting percentage alongside an improvement in overall root system development. The treatment was further associated with the formation of

a greater number of first-order roots, which positively influenced the subsequent performance of planting stock. Importantly, the gel-based formulation was found to minimise the leaching of the active compound, thereby ensuring more consistent conditions for the induction of adventitious roots. It was also emphasised within the study that the effectiveness of rooting stimulants is strongly contingent upon their integration with an optimal substrate and appropriate environmental conditions, particularly temperature regime, air humidity, and the physical properties of the rooting medium.

The effectiveness of auxin-containing formulations has also been confirmed in *ex vitro* systems, where A.D. Oakes *et al.* (2020), analysing rooting in *Castanea dentata*, demonstrated that treatment of shoot bases with Clonex Rooting Gel stimulates adventitious root formation and improves subsequent acclimatisation success, indicating its suitability for enhancing both rhizogenesis and planting material quality. Despite the substantial body of research on the use of growth regulators in the rooting of woody plant cuttings, the effectiveness of rhizogenesis stimulators in the vegetative propagation of *Picea pungens f. glauca* remains insufficiently explored. The aim of this study was to evaluate the effectiveness of rhizogenesis stimulators in the rooting of semi-hardwood cuttings of *Picea pungens f. glauca*.

MATERIALS AND METHODS

The study was conducted during 2023-2025 to determine the effect of different rooting stimulators on the rooting of semi-hardwood cuttings of *Picea pungens* Engelm. f. *glauca*. The experimental methodology was based on a comparative design incorporating both control and treatment variants, with the latter involving the application of different growth regulators.

The experimental design comprised a control treatment without the application of rooting stimulants, in which cuttings were treated with water prior to planting, and a series of experimental variants involving the use of commercial formulations, namely *Clonex Green*, *Clonex Purple*, and *Clonex Red*. The active ingredient in these preparations is IBA, present at concentrations of 1, mg L⁻¹ in Clonex Green, 3,000 mg L⁻¹ in Clonex Purple, and 8,000 mg L⁻¹ in Clonex Red. In addition, aqueous solutions of IBA at concentrations of 1,000, 3,000, and 5,000 mg L⁻¹ were included as separate treatments. The selection of these products was informed by an analysis of recent scientific literature, which highlighted the high efficacy of auxin-based growth regulators in stimulating adventitious root formation in woody plant cuttings (Abshahi *et al.*, 2022; Swathi *et al.*, 2025; Silva Filho *et al.*, 2026). The specific concentrations and formulation types were chosen with due consideration of

the biological characteristics of the study species and the experimental conditions.

Cuttings were collected from stock plants up to 10 years of age. The selection of donor plant age was guided by methodological considerations, as reported by M. Younessi-Hamzekhanlu *et al.* (2026). Cuttings were taken from the upper part of the crown. Shoots of medium thickness were selected for propagation, while both excessively thin and overly thick shoots were excluded, as the morphometric characteristics of cuttings have a significant influence on the intensity of rhizogenesis. In the present study, cuttings measured 8-12 cm in length, with a basal diameter of 0.3-0.5 cm. Cuttings were excised using secateurs without the formation of a "heel". Collection was carried out in spring, in early April, prior to the onset of active shoot growth. The cuttings measured 8-12 cm in length. Following selection, the cuttings were transported to the greenhouse in a moist environment to prevent desiccation.

Prior to planting, needles were removed from the basal 2-3 cm of each cutting in order to reduce the risk of tissue decay and to improve contact between the cutting base and the substrate. The basal ends were then briefly treated with Clonex and aqueous solutions of IBA for 3-5 seconds. Cuttings were inserted into the substrate to a depth of 2-3 cm. The rooting medium consisted of a 1:1 mixture of river sand and high-moor peat. The selection of this substrate was determined by its high aeration capacity, adequate moisture retention, and overall suitability for root system development. Prior to the establishment of the experiment, the substrate was pre-treated to eliminate pathogenic microorganisms and weed seeds. The sand component was thoroughly washed under running water to remove fine dust and silt particles. After thorough mixing, the sand-peat substrate was subjected to thermal treatment by steaming. This procedure was carried out at a temperature of approximately 90-95°C for 30-40 minutes.

The study was conducted under protected ground conditions in a greenhouse, allowing for controlled microclimatic parameters. Air temperature during the

rooting period was maintained within the range of 25-30°C, which is considered optimal for the induction of rhizogenesis in semi-hardwood cuttings of coniferous species. To prevent overheating during the summer period, shading was applied using a polymer shade net, which reduced solar radiation intensity and helped to stabilise the temperature regime. Optimal air and substrate moisture levels were maintained using a fine mist irrigation system. Watering was carried out at 30-minute intervals with a duration of 15 seconds. This regime ensured a consistently high relative humidity of 85-95%, thereby reducing transpiration, preventing cutting desiccation, and creating favourable conditions for the formation of adventitious roots.

The experiment was established using a replicated design. Each treatment comprised 30 cuttings with four replicates, providing a sufficient sample size for subsequent statistical analysis. The total number of cuttings per treatment therefore amounted to 120. Rooting efficiency was assessed by recording the number of cuttings that had formed a root system by the end of the rooting period. Evaluation was conducted 120 days after planting. The study was conducted in accordance with ethical standards for work involving biological materials (Convention on Biological Diversity, n.d.). Statistical analysis of the results was performed using Microsoft Excel, with the data processed as both absolute values and percentages relative to the total number of cuttings in each treatment. The reliability of the obtained data was assessed using analysis of variance (ANOVA), which enabled the determination of the effects of the studied factors on the rooting process of cuttings.

RESULTS AND DISCUSSION

An investigation was conducted to evaluate the effects of different formulations and concentrations of auxin-based treatments on the rooting of semi-hardwood cuttings of *Picea pungens* f. *glauca*. While a graphical representation of the obtained results expressed as percentages is shown in Figure 1.

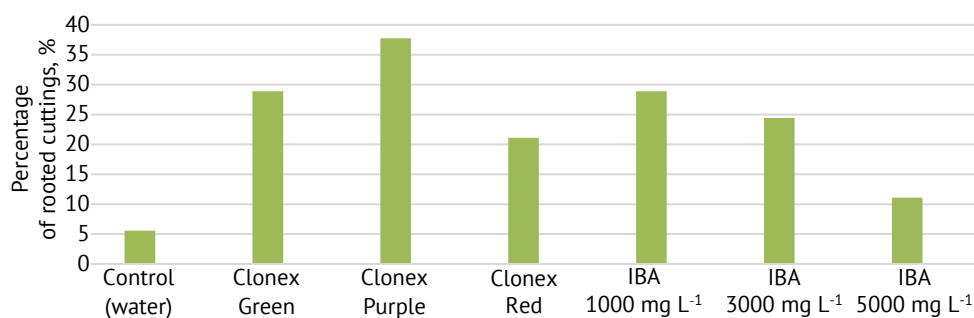


Figure 1. Comparative effectiveness of Clonex preparations and IBA concentrations on the rooting of *Picea pungens* f. *glauca* cuttings (average for 2023-2025)

Source: developed by the authors

The control treatment, in which the cuttings had been planted without the application of growth stimulators, showed the lowest rooting level throughout the entire study period: in 2023 only one cutting rooted, while in 2024 and 2025 two cuttings rooted each year. On average over the three-year period, only 1.67 cuttings rooted out of the total number established per treatment, which corresponds to 5.6%. This confirmed the low natural ability of *Picea pungens* f. *glauca* cuttings to form roots without the use of rhizogenesis stimulators. In the treatment with Clonex Green, 9 cuttings rooted in 2023, 10 cuttings rooted in 2024, and 9 cuttings rooted in 2025. For comparison, in the control treatment only 1, 2, and 2 cuttings rooted during the same years. Thus, even with a minimal exposure time of the preparation (3-5 s), the use of Clonex Green ensured a 4-5-fold higher rooting level compared with the control. The average number of rooted cuttings over the three-year period was 8.67, which corresponded to 28.9%, which indicates the consistently high effectiveness of this preparation.

The highest rooting values were obtained with the application of Clonex Purple. In 2023, 12 cuttings rooted; in 2024, 11 cuttings rooted; and in 2025, 11 cuttings rooted. In comparison with the control treatment, where only 1-2 cuttings rooted, this preparation ensured a substantially higher rooting level. The average number of rooted cuttings during the study period was 11.3, corresponding to 37.8%, which allowed Clonex Purple to be considered the most effective among the tested preparations. The high efficacy of Clonex Purple was attributed to both its composition and physical form. The gel-based formulation ensured uniform coverage of the cutting base and prolonged contact between the active substance and plant tissues. This facilitated the gradual penetration of the growth regulator into cambial and parenchymal tissues, thereby stimulating the initiation of root primordia. The combination of an IBA (optimal

auxin) concentration and sustained contact with the cutting surface resulted in the highest rooting percentage (37.8%) being recorded in the treatment involving Clonex Purple. The application of Clonex Red proved to be less effective. In 2023, 6 cuttings rooted; in 2024, 7 cuttings rooted; and in 2025, 6 cuttings rooted. Although these values significantly exceeded those of the control treatment, they were lower than the results obtained with the other Clonex preparations. On average over the three years, 6.3 cuttings rooted, corresponding to 21.1%.

The analysis of treatments involving the application of IBA showed that the most effective among the tested concentrations was 1,000 mg L⁻¹. In 2023 and 2024, 9 cuttings rooted in this treatment each year, while in 2025, 8 cuttings rooted, which was significantly higher than the control. The average result over the three-year period was 8.67 rooted cuttings, corresponding to 28.9%.

In the treatment with an IBA concentration of 3,000 mg L⁻¹, 8 cuttings rooted in 2023, 7 cuttings in 2024, and 7 cuttings in 2025. Compared with the control, these values were also considerably higher; however, they were somewhat lower than those obtained with the concentration of 1,000 mg L⁻¹. The average number of rooted cuttings in this treatment was 7.3, corresponding to 24.4%.

The lowest results among the IBA treatments were recorded at the concentration of 5,000 mg L⁻¹. In 2023, 3 cuttings rooted; in 2024, 3 cuttings rooted; and in 2025, 4 cuttings rooted. Although these values still exceeded those of the control treatment, they were substantially lower compared with the other IBA concentrations, indicating an inhibitory effect of the excessive auxin concentration on root formation processes. The average rooting value in this treatment was only 3.3 cuttings, corresponding to 11.1%. The summarised quantitative indicators of cutting rooting over the three-year study period are presented in Table 1.

Table 1. Rooting percentage of *Picea pungens* f. *glauca* cuttings as influenced by Clonex formulations and IBA concentration (2023-2025)

Treatment variant	Concentration mg L ⁻¹	Number of cuttings in the treatment, pcs.	Rooted cuttings, pcs. (2023)	Rooted cuttings, pcs. (2024)	Rooted cuttings, pcs. (2025)	Average number of rooted cuttings in 2023-2025, pcs.
Control (water)	water	30	1	2	2	1.67
Clonex Green	1,500	30	9	8	9	8.67
Clonex Purple	3,000	30	12	11	11	11.33
Clonex Red	8,000	30	6	7	6	6.33
IBA	1,000	30	9	9	8	8.67
IBA	3,000	30	8	7	7	7.33
IBA	5,000	30	3	3	4	3.33
LSD_{0.05}			1.71	1.83	1.76	

Source: developed by the authors

Despite the higher concentration of the active substance in Clonex Red (8,000 mg L⁻¹), its application proved to be more effective than the aqueous IBA solution at a concentration of 5,000 mg L⁻¹. This was attributed to the sustained release of auxin from the gel-based formulation, which ensured a more gradual and controlled effect on cutting tissues, whereas high concentrations of IBA in aqueous solution tended to induce phytotoxic effects and inhibit root formation processes. The decline in rooting efficiency observed at increased IBA concentrations of 3,000 and 5,000 mg L⁻¹ was associated with the phytotoxic effects of excessive auxin doses. An overabundance of growth regulators might disrupt the normal course of physiological processes within cutting tissues, leading to the inhibition of cell division and differentiation. At high concentrations, auxins could also induce the formation of necrotic zones in the basal region and disrupt the balance of endogenous phytohormones. As a result, instead of stimulating rhizogenesis, a partial suppression of root system formation occurred, as evidenced in the treatments with 3,000 and 5,000 mg L⁻¹.

Using the analysis of variance (ANOVA) method, mathematical processing of the experimental data confirmed the statistical significance of the effect of the studied growth stimulators (Clonex preparations and different concentrations of IBA) on the intensity of root system formation in *Picea pungens* f. *glauca*. The calculated least significant difference (LSD_{0.05}) values were 1.71 in 2023, 1.83 in 2024, and 1.76 in 2025, which clearly demonstrated a significant increase in rooting rates in the treatments with Clonex Purple, Clonex Green, and the IBA solution at a concentration of 1,000 mg L⁻¹ compared with the control, thus excluding the influence of random experimental error. A significant improvement over the control was observed with the application of Clonex Green, Clonex Purple, and Clonex Red, as well as with aqueous IBA solutions at concentrations of 1000, 3000, and 5000 mg L⁻¹ over the three-year study period. An exception was recorded in 2024, when the application of IBA at 5000 mg L⁻¹ did not result in a statistically significant advantage over the control.

Overall, the results obtained indicate the expediency of using auxin-containing formulations in the vegetative propagation of *Picea pungens* f. *glauca*, as their application substantially enhances the rooting efficiency of cuttings. Among the treatments studied, Clonex Purple demonstrated the highest effectiveness. The use of gel-based stimulants may therefore be recommended for ornamental horticulture practice, as they provide more stable conditions for the action of growth regulators and contribute to higher rooting percentages.

It should also be noted that the rooting efficiency of cuttings may have been influenced by environmental conditions, particularly temperature, air humidity, and light intensity within the greenhouse. Optimal temperature and elevated humidity levels contribute to reduced transpiration and prevent desiccation of cuttings during the period of root system formation. At the same time, an adequate level of diffused light supports photosynthetic activity in the tissues, promoting the accumulation of assimilates required for the development of adventitious roots. Thus, the combination of optimal microclimatic conditions with the use of effective rhizogenesis stimulants creates favourable prerequisites for increasing the rooting percentage of *Picea pungens* f. *glauca* cuttings.

The results of the present study were consistent with the findings reported by M.F. El-Banna *et al.* (2023), who investigated the influence of IBA on the formation of adventitious roots in cuttings of *Zanthoxylum beecheyanum*. In that study, it was established that the application of exogenous auxin stimulated the rhizogenesis process, resulting in an increase in rooting percentage, as well as in the number and length of roots. In particular, rooting in the control treatments ranged from approximately 20-25%, whereas treatment with IBA at a concentration of 500 mg kg⁻¹ increased this value to 40-45%. The application of a concentration of 1,000 mg kg⁻¹ increased the rooting percentage to 55-60%, while concentrations of 1,500-2,000 mg kg⁻¹ resulted in 70-80% of rooted cuttings. However, a further increase in concentration to 2,500 mg kg⁻¹ was accompanied by a decline in rooting efficiency. The results obtained in the study likewise confirmed the pronounced stimulating effect of auxin-containing preparations on the formation of adventitious roots, demonstrating optimal efficiency at lower concentrations and a decline at higher concentrations.

Comparable findings regarding the stimulatory effect of auxin-based formulations on the rooting of coniferous cuttings have been reported by J.B. Silva Filho *et al.* (2026), who, in the course of clonal propagation of *Pinus lambertiana* and *Pinus ponderosa*, applied a gel-based Clonex preparation containing IBA at a concentration of approximately 0.31%. It was established that the use of this formulation resulted in high rooting performance: in *Pinus lambertiana*, the proportion of rooted cuttings reached 80%, whereas in *Pinus ponderosa* it was approximately 40%, significantly exceeding the control treatments, where rooting ranged from 10% to 30%. The results were consistent with this trend, confirming that the gel-based formulation (Clonex Purple) provides superior rooting performance compared to the control and aqueous solutions.

The difference in absolute values can be attributed to species-specific characteristics.

Important results regarding the role of stimulators have also been obtained in studies on the rooting of *Cupressus macrocarpa* cuttings. In the study by Y.A. Abdulraahman & M.Z. Ayoub (2023), it was established that the application of auxin-based growth regulators significantly improved the rooting of semi-hardwood cuttings of this species. The maximum rooting percentage in their experiment reached 51.11%, whereas the control treatment ranged from 26% to 28%, depending on the experimental conditions. The present findings similarly confirmed the positive influence of auxin-based preparations on the formation of adventitious roots. As observed in experiment, the maximum rooting efficiency was achieved at lower IBA concentrations and with the Clonex Purple, while higher doses led to a decline.

The lower rooting level observed in comparison with the results reported for *Cupressus macrocarpa* might be explained by interspecific differences among the studied plants. Species of the genus *Picea* are generally characterised by a lower capacity for adventitious root formation compared with many other conifer species. This is associated with the anatomical characteristics of their shoots, a higher degree of tissue lignification, and lower activity of cambial cells involved in the formation of root primordia. In the study by M. Sanz Gallego *et al.* (2025), it was found that the application of IBA at a concentration of 4,000 ppm significantly increased the efficiency of rhizogenesis in cuttings of *Juniperus communis*. The maximum rooting percentage reached 45.6%, whereas in the control treatment it was 23.5%. These results confirmed the important role of exogenous auxins in stimulating the formation of adventitious roots in conifer species. A comparable positive response to the application of IBA and the commercial preparation Clonex was observed in the present study. These differences in results may be attributed to interspecific characteristics of the studied plants.

The obtained results were appropriately compared with the data reported by G.E. Oğuztürk *et al.* (2025), who investigated the effects of different auxin types (IBA, NAA, IAA) at concentrations of 1,000; 3,000; 5,000; and 8,000 ppm on the rooting of semi-hardwood cuttings of *Photinia × fraseri*. In that study, rhizogenesis exhibited a clearly defined concentration optimum, with the highest rooting percentage ($93.33 \pm 8.16\%$) achieved at an IBA concentration of 5,000 ppm, significantly exceeding the control ($58.33 \pm 7.53\%$). At the same time, a reduction in concentration to 1,000 ppm resulted in decreased rooting ($48.33 \pm 11.69\%$), whereas an increase to 8,000 ppm led to inhibition of the rooting process.

The present findings demonstrated a comparable concentration-dependent pattern, characterised by the presence of an optimal level and the suppression of rhizogenesis when this threshold is exceeded. At the same time, it should be noted that the effective auxin concentrations reported in the cited study were substantially higher than those observed in the present work. This discrepancy is attributable to species-specific characteristics and differences in physiological sensitivity to auxins. This was further illustrated by the control treatments, where the rooting percentage in the present study was 5.6%. Coniferous species, including *Picea pungens* f. *glauca*, are generally characterised by a higher sensitivity to exogenous growth regulators, which consequently narrows the effective range of their application.

The effects of indole-3-butyric acid (IBA) concentrations (4,000; 6,000; and 8,000 mg L⁻¹) and substrate composition on the rooting of semi-hardwood cuttings of *Photinia × fraseri* were investigated by N.S. Hasan & Y.H. Hammo (2021). The study demonstrated that the application of IBA significantly enhanced rooting performance: at a concentration of 4,000 mg L⁻¹, the rooting percentage reached 43.33%, while at 6,000 and 8,000 mg L⁻¹ it increased to 50.56% and 51.78%, respectively, compared with substantially lower values in the untreated control. Moreover, when peat was used as the substrate in combination with 6,000 mg L⁻¹ IBA, rooting increased to 73.33%, highlighting the critical role of interactions between environmental factors and growth regulators in the rhizogenesis process. The present results were consistent with these findings, confirming the stimulatory effect of IBA on cutting rooting, albeit with a decline at higher concentrations. This discrepancy was attributed to species-specific characteristics, as well as differences in the physiological status of the cuttings and their sensitivity to exogenous auxins. In addition, the higher rooting performance observed with Clonex formulations compared with aqueous IBA solutions underscored the importance of the preparative form, which ensured a prolonged release of auxin and a more effective realisation of its physiological action.

The influence of different concentrations of indole-3-butyric acid (0-6,000 ppm) on the rooting of *Corylus colurna* cuttings under controlled greenhouse conditions was investigated by S. Çolak *et al.* (2025). The study demonstrated that the highest rooting percentage (25%) was achieved at an IBA concentration of 5,000 ppm, whereas at 1,000; 2,000; and 6,000 ppm rooting remained at approximately 15%. The lowest value (8.3%) was recorded at 4,000 ppm. In addition, the greatest number of roots per cutting (on average 8) was observed at 1,000 ppm, compared with only 0.6 in

the control, indicating a pronounced stimulatory effect of auxin on root system morphogenesis. The present results partially corresponded to these findings, as they also confirmed the existence of an optimal concentration range of IBA for the stimulation of rhizogenesis. However, in contrast to the cited study, where the optimum was shifted towards higher concentrations (5,000 ppm), lower auxin concentrations proved to be more effective for *Picea pungens* f. *glauca*. This difference was attributable to species-specific characteristics and an increased sensitivity of this taxon to exogenous growth regulators.

Furthermore, the use of Clonex formulations resulted in higher rooting performance compared with aqueous IBA solutions, which highlighted the important role of the preparative form in enhancing auxin effectiveness. Overall, the findings supported the species-specific nature of optimal auxin concentrations and emphasised the need to consider both concentration and formulation when stimulating rhizogenesis. The application of indole-3-butyric acid at concentrations of 0; 1,000; 2,000; and 3,000 mg L⁻¹ in combination with substrate composition during the rooting of hardwood cuttings of *Cydonia oblonga* was examined by R.R. Aziz *et al.* (2024). The study revealed that IBA application did not enhance rhizogenesis efficiency: rooting percentages under auxin treatment ranged from 46% to 47%, and in some cases decreased to 7-8% depending on the substrate. In contrast, the control treatment, involving cultivation in pure river sand without auxin application, achieved a rooting percentage of 70.83%. These findings indicated the absence of a positive effect of IBA for this species within the tested concentration range.

The present results differed markedly from these observations, as a clear stimulatory effect of both aqueous IBA and Clonex preparations on the rooting of *Picea pungens* f. *glauca* was established, significantly exceeding the control. The observed discrepancies can be attributed to both species-specific characteristics and differences in rooting conditions. Unlike studies in which the highest rooting performance was achieved in pure river sand without auxin application, the present experiment utilised a substrate composed of a 1:1 mixture of river sand and peat, characterised by improved moisture retention and aeration properties. These conditions likely enhanced the effectiveness of exogenous auxin and promoted the formation of adventitious roots. Thus, the efficiency of IBA application was determined not only by species-specific traits but also by the complex of rooting conditions, particularly substrate composition.

The results can be appropriately compared with those reported by K. Harika *et al.* (2024), who investigated the effects of different concentrations of

indole-3-butyric acid (0; 1,000; 1,500; and 2,000 ppm) on the rooting of semi-hardwood cuttings of *Punica granatum*. In that study, the highest rooting percentage (58.89%) was achieved at an IBA concentration of 2,000 ppm, while the control treatment showed substantially lower values. Moreover, this concentration resulted in the greatest number of roots per cutting (12.02) and the maximum root length (14.14 cm), indicating a pronounced stimulatory effect of auxin on rhizogenesis.

The obtained results were consistent with those reported in the cited study and confirmed the positive effect of IBA on cutting rooting. At the same time, it was established that the optimal concentration for *Picea pungens* f. *glauca* is lower than that reported in the referenced work, indicating a greater sensitivity of this taxon to exogenous auxins. However, in contrast to the cited study, where the optimum was observed at a higher concentration (2,000 ppm), increasing the IBA concentration to 3,000 and 5,000 mg L⁻¹ in *Picea pungens* f. *glauca* resulted in a decline in rooting efficiency (to 24.4% and 11.1%, respectively), indicating a greater sensitivity of this species to excessive auxin levels. This was attributed to species-specific characteristics of coniferous plants and a more pronounced manifestation of phytotoxic effects when the optimal concentration of the growth regulator is exceeded. Thus, the results confirmed that the effectiveness of IBA is determined not only by its presence but also by the precise selection of concentration and the preparative form of application.

The effects of indole-3-butyric acid (IBA) at concentrations of 0; 1,000; 2,000; and 3,000 mg L⁻¹ in combination with substrate composition on the rooting of *Bougainvillea* spp. cuttings were investigated by A.T. Rasul *et al.* (2025). The study demonstrated that the untreated control consistently exhibited the lowest rooting performance regardless of substrate type. Application of IBA at 1,000 mg L⁻¹ increased rooting percentage and stimulated shoot development, although it did not produce maximum values. The highest rooting level (up to 80.49% in *Bougainvillea glabra*) was achieved at 2,000 mg L⁻¹, which was identified as the optimal concentration for the induction of adventitious roots. Further increasing the concentration to 3,000 mg L⁻¹ did not enhance rooting percentage but promoted the development of a more robust root system, including greater root number and length. In addition, peat as a substrate provided the most favourable conditions for rooting compared with other media.

The present findings partially corresponded to these results, confirming the stimulatory effect of IBA on rhizogenesis. However, in contrast to the cited study, the optimal concentration range appeared to be narrower for *Picea pungens* f. *glauca*. Furthermore, whereas

substrate composition played a significant role in the cited study, the present results indicated a more pronounced effect of auxin concentration and formulation. In particular, the use of Clonex preparations ensured higher rooting performance compared with aqueous IBA solutions. Overall, the findings confirmed that rhizogenesis efficiency is governed by a complex interaction of factors, among which both the concentration of the growth regulator and the rooting conditions are of primary importance.

The obtained results can be compared with those reported by F.E.F. de Felice *et al.* (2024), who investigated the effect of different concentrations of indole-3-butyric acid (0; 1,000; 2,000; and 4,000 mg L⁻¹) on the rooting of mini-cuttings of *Eucalyptus camaldulensis* collected under arid conditions. The study demonstrated that the application of IBA significantly enhanced rhizogenesis: rooting in the control reached 55%, whereas treatment with 2,000 mg L⁻¹ IBA increased this value to 80%, representing the optimal response. In contrast, concentrations of 1,000 and 4,000 mg L⁻¹ did not result in further improvement and were inferior to the optimal treatment, indicating a clearly defined auxin optimum.

The present findings were generally consistent with these data, as they likewise confirmed the dependence of rooting efficiency on IBA concentration. Similar to the cited study, the existence of an optimal concentration range was identified, beyond which rhizogenesis efficiency decreases. At the same time, although the presence of an auxin optimum was confirmed, the absolute values of rooting differ substantially, being markedly lower for *Picea pungens* f. *glauca*. This discrepancy was attributable to biological characteristics of coniferous species, which generally exhibit a lower capacity for adventitious root formation compared with representatives of the genus *Eucalyptus*.

A comparative analysis of the obtained results with the data reported by I.S. Negri *et al.* (2025) indicated that their study examined the effects of indole-3-butyric acid concentration and the age of donor plants on the rooting of *Fraxinus excelsior* cuttings. It was established that cuttings collected from juvenile plants exhibited a rooting percentage of 39.44% without the application of stimulants, whereas material derived from mature trees failed to form roots. In treatments involving IBA at concentrations of 1-2%, rooting of juvenile cuttings ranged from 44.82% to 58.62% after 6 months of cultivation, increasing further to 58.62-75.86% after 9 months, demonstrating the enhanced effectiveness of auxin when physiologically young material is used.

The present findings partially corresponded to these results, as a stimulatory effect of IBA on the rooting

of *Picea pungens* f. *glauca* cuttings was likewise observed, although the overall level of rhizogenesis was substantially lower. This discrepancy appeared to be systematic and could primarily be explained by interspecific differences, as representatives of the genus *Picea* are characterised by a considerably lower morphogenetic potential for adventitious root formation compared with deciduous species such as *Fraxinus excelsior*. In addition, the cited study highlighted the critical role of donor plant age, whereas in the present work cuttings were obtained from plants up to 10 years old, which partially mitigates this factor but does not allow similarly high rooting performance to be achieved. Furthermore, the application of Clonex Purple increased rooting to 37.8%. However, even this value remained lower than those reported for *Fraxinus excelsior*. Overall, these findings indicated that the effectiveness of auxin-based treatments was determined not solely by concentration but by a complex interplay of species-specific traits, physiological status of the source material, and rooting conditions, which may lead to substantial variation among studies.

Important findings on the effect of indole-3-butyric acid on rhizogenesis in coniferous cuttings were reported by Abshahi *et al.* (2022), who investigated the influence of IBA concentration, substrate type, and cutting season on the rooting of *Juniperus sabina*. The study employed a wide range of IBA concentrations (0, 1000, 2000, 4000, and 8000 ppm) in combination with different substrates and seasonal timings of cutting collection. It was demonstrated that IBA treatment significantly increased the rooting percentage, root biomass, and morphological characteristics compared with the control. The maximum rooting rate reached 62% at a concentration of 1000 ppm. The results obtained are broadly consistent with these findings, as both studies revealed a clearly expressed stimulatory effect of IBA on adventitious root formation. In particular, the increase in rooting percentage under the influence of exogenous auxin, relative to the control, confirmed the key role of IBA as a regulator of cellular differentiation and the initiation of root primordia.

At the same time, notable differences were identified in both the level of effectiveness and the optimal concentrations. In the cited study, rooting percentages exceeded 60%, whereas in the case of *Picea pungens* f. *glauca*, the maximum value reached 37.8% with Clonex Purple and 28.9% with an aqueous IBA solution (1000 mg L⁻¹). Moreover, in *Juniperus sabina*, the effective IBA concentration range was broader (1000-4000 ppm), while in the present study, increasing the concentration to 3000-5000 mg L⁻¹ resulted in reduced rooting, indicating a narrower optimum and a higher

sensitivity of this taxon to auxin. These differences can be attributed to species-specific biological characteristics. Representatives of the genus *Picea* generally exhibit a lower capacity for adventitious root formation compared with those of the genus *Juniperus*, which is associated with a higher degree of tissue lignification and lower cambial activity. In addition, the cited study demonstrated a substantial influence of seasonal timing and substrate type on rhizogenesis, whereas in the present work these factors were standardised under controlled experimental conditions.

The results of the present study are consistent with the generalisations reported in the review by Younesi-Hamzekhanlu *et al.* (2026), which synthesises current approaches to the vegetative propagation of species within the genus *Picea*. The authors demonstrated that the efficiency of rhizogenesis is governed by a complex interaction of endogenous and exogenous factors, among which the age of stock plants, the physiological status of cuttings, genotype, and environmental conditions are of primary importance. It was shown that cuttings derived from juvenile plants exhibit a substantially higher rooting capacity compared with material obtained from mature trees, which is associated with higher metabolic activity and increased sensitivity to exogenous auxins.

In the present study, cuttings were collected from plants up to 10 years of age, which enabled root system formation in *Picea pungens* f. *glauca* and confirmed the importance of using physiologically juvenile donor material. At the same time, the recorded rooting percentages (maximum 37.8%) remained moderate, which is in agreement with the general conclusion of the cited authors regarding the relatively low inherent capacity of *Picea* species for adventitious root formation. The findings of the review further indicate that the effectiveness of auxins in rhizogenesis depends not only on their application per se, but also on their concentration, as both insufficient and excessive doses may reduce rooting or exert inhibitory effects. In addition, the review emphasised the importance of interactions between growth regulators and environmental conditions, particularly substrate composition and moisture regime. This was consistent with the results obtained, where the use of a sand-peat substrate (1:1), in combination with controlled greenhouse conditions, provided a favourable environment for the action of auxins and the development of adventitious roots.

CONCLUSIONS

As a result of the conducted research, it was established that the rooting of semi-hardwood cuttings of

Picea pungens Engelm. f. *glauca* largely depends on the application of rooting stimulators. In the control treatment without the use of growth regulators, the average rooting percentage was only 5.6%, indicating the low natural capacity of cuttings of this ornamental form to produce adventitious roots. The application of auxin-containing preparations significantly increased the rooting efficiency of the cuttings. The highest values were obtained with the use of Clonex Purple, where the average rooting percentage reached 37.8%. Slightly lower but still relatively high results were recorded in the treatments with Clonex Green and an aqueous solution of IBA at a concentration of 1,000 mg L⁻¹, where the average rooting level was 28.9%. The use of Clonex Red provided an average rooting percentage of 21.1%.

It was also established that the effectiveness of IBA largely depends on its concentration. Increasing the concentration to 3,000 mg L⁻¹ resulted in a decrease in the rooting level to 24.4%, whereas the use of a concentration of 5,000 mg L⁻¹ caused a sharp decline in the number of rooted cuttings, which amounted to 11.1%. This reduction may be explained by the phytotoxic effect of excessive auxin concentrations on the tissues of the cuttings. Analysis of variance (ANOVA) demonstrated that, over the three-year study period, all treatments showed a statistically significant advantage over the control, with the exception of the treatment involving IBA at a concentration of 5,000 mg L⁻¹ in 2024.

The obtained results indicate that the use of Clonex preparations based on IBA in gel form is more effective for stimulating rhizogenesis in cuttings of *Picea pungens* f. *glauca*, as this formulation ensures a more uniform distribution of the active substance on the cut surface and provides a prolonged effect of the growth regulator. Prospects for further research include the improvement of vegetative propagation techniques for *Picea pungens* f. *glauca*, particularly through the study of substrate composition and the combined application of growth regulators with other biologically active substances in order to increase rhizogenesis efficiency and obtain high-quality planting material.

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Вплив препарату Clonex та індоліл-3-масляної кислоти на ризогенез живців *Picea pungens f. glauca*

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Анотація. Метою дослідження було кількісне оцінювання впливу різних концентрацій індоліл-3-масляної кислоти та гелевих препаратів на інтенсивність ризогенезу напівздерев'янілих живців *Picea pungens f. glauca* з визначенням найбільш ефективних варіантів обробки. У трирічному дослідженні (2023-2025 рр.) досліджено ефективність препаратів Clonex Green, Clonex Purple, Clonex Red, а також водних розчинів індолілмасляної кислоти (ІМК) у концентраціях 1000 мг/л, 3000 мг/л та 5000 мг/л при розмноженні напівздерев'янілих живців *Picea pungens f. glauca*. Живці довжиною 8-12 см заготовляли з маточних рослин віком до 10 років. Живці висаджували в субстрат, який складався із суміші річкового піску та верхового торфу у співвідношенні 1:1. За результатами досліджень встановлено, що застосування препаратів Clonex та ІМК істотно підвищує відсоток укорінення порівняно з контрольним варіантом, де середній показник становив лише 5,6 %. Найвищу ефективність продемонстрував гелевий препарат Clonex Purple, використання даного препарату забезпечило укорінення живців 37,8 %. Високу результативність (28,9 %) також зафіксовано при застосуванні Clonex Green та водного розчину ІМК у концентрації 1000 мг/л. Виявлено, що подальше підвищення концентрації чистої ІМК до 3000 мг/л призводить до зниження показника до 24,4 %, а застосування концентрації ІМК 5000 мг/л спричиняє пригнічення ризогенезу (11,1 %), що свідчить про фітотоксичну дію. Встановлено перевагу гелевих форм стимуляторів (Clonex) над водними розчинами, що пояснюється пролонгованим контактом діючої речовини з основою живця, її поступовим вивільненням та захистом від вимивання із субстрату. Математична обробка даних методом дисперсійного аналізу підтвердила статистичну достовірність отриманих результатів (НІР0,05 у межах 1,71-1,83). Отримані дані дозволяють рекомендувати оптимальні концентрації стимуляторів для підвищення виходу якісного садивного матеріалу декоративних хвойних порід

Ключові слова: вегетативне розмноження; укорінення рослин; ауксини; адвентивні корені; стимулятори коренеутворення