Effectiveness of stimulating treatment cuttings of privet common (*Ligustrum vulgare* L.) with biologically active preparations

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**Abstract.** The effectiveness of the use biologically active substances in the rooting of cuttings privet common (*Ligustrum vulgare* L.) in its introduction in the region of Nizhny Novgorod was studied. The processes of callus adventitious root formation were studied. The research target was an arboretum collection of common privet in Nizhny Novgorod State Agricultural Academy with geographical coordinates 56°19’43” N, 44°00’07” E and an altitude of 141 m above sea level. Tested nine industrially produced biologically active preparations widely used in tree and forest nurseries to increase the efficiency of rooting cuttings. The positive effect of cutting treatment with biologically active preparations was confirmed. A significant intensity of callus formation was recorded in the variants with profistim (98.50±1.05%) and extrasol (96.50±0.76%). A noticeable increase in the number of adnexal roots was caused by the use of the drugs heteroauxin (24.55±1.08 pcs.) and ferovit (22.00±1.38 pcs.). The best results for the total length of adventive roots were obtained after the use of heteroauxin (266.92±16.71 cm) and ecofus (272.22±14.42 cm). The effectiveness of bioactive drugs in stimulating regeneration processes on privet cuttings, increasing in the intensity of callus formation and the rate of linear growth of adventitious roots has been proven.

1. Introduction

Improving the assortment of urban green spaces is in the context of the strategic objectives of optimizing the basic environmental parameters of modern urban ecosystems. To a large extent, it is associated with the need to involve introduction methods to solve these problems, which allow us to significantly expand the list of tree and shrub species that can successfully perform sanitary and hygienic, decorative and aesthetic, and recreational and balneological functions. Among them, with good reason, it’s possible to include the *privet common* (*Ligustrum vulgare* L.), which is an exotic in the Middle Volga region, where it is of interest in landscaping forest-reclamation and protective plantings of various designs. Progressive technologies for cloning woody and shrubby plants are of crucial importance because they permit to expand the range of its economic application and further distribution in culture on the territory of the Russian Federation and in particularly in the region of Nizhny Novgorod. Especially it’s important when replicating their forms and varieties.

In Europe, the common privet is involved in the processes of vegetation succession occurring on the displaced ancient forest soil [1]. In some cases, it is considered to be a potentially invasive species [2]. Cuttings are traditionally considered as a productive method of vegetative propagation of a wide
range of trees and shrubs [3,4], in particular, oak [5], maple [6], chestnut [7], fir [8], willow [9], poplar [10,11], plum [12], which has not lost its relevance today. In this regard, it’s accepted that further researches of its numerous technological and biological aspects are expedient. The study of the reaction of cuttings, in particular of landscape shrubs cuttings, to the introduction of biologically active components into the substrate [13,14] or the reaction of plants to the rooting of cuttings in different types of substrates [11] continues. A comprehensive analysis of the growth and development of the aboveground part and root systems of cuttings is carried out [15,16], including the processes of callus formation and rhizogenesis occurring on cuttings [17]. A promising means of increase in the efficiency of vegetative reproduction is the use of various growth regulators and stimulators of regenerative processes [18-20]. Introduction as one of the spheres of human economic activity and one of the most relevant areas of scientific research is of undoubted interest to forestry specialists [21,22]. Certain successes have been achieved in the region of Nizhny Novgorod. One of the stages of our work is the introduction and domestication of the common privet. In this context, the aim of the research is to give a comparative assessment of the effectiveness of the use of biologically active drugs to stimulate rhizogenesis and post-regenerative development of root systems in privet common and to identify the most effective of them.

2. Methods and materials

The research target was the collection plantings of privet common (Ligustrum vulgare L.), located on the territory of the arboretum of Nizhny Novgorod State Agricultural Academy. It’s geographical coordinates are 56°19'43" N, 44°00'07" E, it’s altitude is 141 m above sea level. According to the forest-growing zoning, its territory is included in the zone of coniferous-broad-leaved forests, in the area of coniferous-broad-leaved (mixed) forests of the European part of the Russian Federation. The methodological basis of the work was the basic requirements for the organization of experience (its typicality, suitability, expediency and reliability), as well as compliance with the principle of the only logical difference [23]. In this context, the selection of accounting plants from the available composition is carried out in a randomized manner. From each of them during the growing season (June 2019), at the same time, physiologically active green cuttings were harvested from the periphery of the middle tier of a well-lit section of the crown for subsequent stimulating processing and rooting. The harvesting was executed from the periphery of the middle tier of a well-lit section of the crown. Abnormally developed shoots or their fragments with signs of damage by external factors were rejected.

The vegetation structures of the experiment are represented by summer greenhouses coated with polyethylene. They are equipped with an automatic fine-dispersed irrigation system. The environmental parameters were controlled with use of METEOSCAN PRO 929 RST02929 automatic weather station. The environmental conditions in the greenhouse (temperature background, illumination, irrigation, substrate characteristics, etc.) were identical in all the tested options.

The number of cuttings prepared for this experiment was 400 pieces. Their length was measured with a ruler with an accuracy of 1 mm; the diameter of the root neck was measured with an electronic caliper (Digital Caliper SH20) with an accuracy of 0.01 mm. The following drugs as options of the experiment were tested: heteroauxin – indolyl-3-acetic acid potassium salt – 50 g/kg (powder); kornevin – 4-(indole-3-yl) butyric acid – 5 g/kg (powder); profistim – is a complex of biologically active substances obtained as a result of deep processing of chicken manure by the method of microbiological synthesis: humic and fulvic acids, phytohormones, vitamins, amino acids, macro- and microelements (solution); siliplant – is a silicon-containing fertilizer, which, in addition to silicon Si (7% from the volume) and potassium (1% from the volume), includes trace elements (mg/l) in an easily accessible chelated form for plants: Fe – 300; Mg – 100; Cu – 70-240; Zn – 80; Mn – 150; Co – 15; B – 90 (solution); ferovite – total nitrogen – 40 g/l, iron (chelate) – 75 g/l (solution); zircon – 0.1 g/l of hydroxycinnamic acids (solution); ecofus – is a liquid organomineral fertilizer based on brown seaweed (Fucus vesiculosus), which contains nitrogen 1.8% from the volume, including in the amide form (NH₂), phosphorus (P₂O₅) 1.0% from the volume, potassium (K₂O) 2.0%
from the volume and more than 40 trace elements, including (g/l): Fe – 1.8; Mg – 0.5; Mn – 1.2; Cu – 0.3; B – 0.4; Zn – 0.3; Ca – 0.25; Mo – 0.2; Co – 0.1; and also Se, I, Si (solution); extrasol – is a strain of rhizospheric, nitrogen-fixing bacteria Basillus subtillus H-13 and their metabolites (solution); epin – 24-epibrassinolide (0.025 g/l) (solution). All the listed preparations are produced by Russian manufacturers and were assigned to the list of biologically active substances tested according to the criterion of their widest industrial use in tree nurseries and similar farms of the Nizhny Novgorod region. There were 9 such drugs. The experiment was controlled by water, in which the cuttings were kept for 18 hours, as well as in solutions, prepared according to the instructions, indicated on the package of the drug. Coarse-grained river sand was used as a substrate. From the fifth day after planting, the biological state of the cuttings was reviewed daily and the effectiveness of the processes of callusogenesis and rhizogenesis occurring in their basal part was evaluated. The final registration of the formed callus was carried out after 30 calendar days. For this period, adventitious roots in one or another quantity and with their different lengths were already found in all the planted cuttings. The final accounting of the effectiveness of rhizogenesis was carried out according to a set of estimates of the length of different categories of roots after the complete completion of the growing season and the transition of average daily temperatures through 0 °C. To make a more complete characterization of the regeneration processes occurring on the lower cuttings as well as to make a comprehensive assessment of the development balance between their root systems and the aboveground part, along with the attributes of direct accounting and direct fixation, we used derived attributes traits that are widely used for this purpose in forestry and biological research [24-26]. In the statistical processing of actual data, including the analysis of variance (ANOVA), generally accepted approaches were used [3,23] with the calculation of descriptive statistics of the criteria of their reliability and reliability according to the Student's T-criterion with the Fisher calculation criterion, the smallest significant difference and the Tukey D-test indicators.

3. Results and discussion

Different effectiveness of stimulating biological preparations in rooting of green (summer) privet cuttings was established. It manifested in different values of some indicators characterizing the regeneration processes occurring on the lower sections of cuttings, such as callus formation and rhizogenesis (table 1).

Thus, according to the intensity of callus formation (table 1) the highest average values were registered in the options of using the drugs profistim (98.50±1.05%) and extrasol (96.50±0.76%), which was by 20% or 1.25 times and by 18% or 1.23 times higher than that in the control version, respectively. Three other drugs showed fairly high results that exceeded the control: heteroauxin-93.50±1.46% (by 15 % or 1.19 times); siliplant-92.00±2.18% (by 13.5% or 1.17 times); 5) ferovit – 94.00±1.78% (by 15.5% or 1.20 times higher). At the same time, some reagents caused a more or less inhibitory effect, which was expressed in a decrease in the callus formation index in comparison with that of the control: zircon – 43.00±4.82% (by – 35.5% or 0.55 times lower; epin – 71.00±3.47% (by – 7.5% or 0.90 times lower). These results were shown on the background of a generalized value for the entire data set (option Total), which, amounting to 84.50±1.17%, exceeded that of the control by 6 % or 1.08 times, which indicates a positive, in general, effect of biologics on the intensity of callus formation on the lower sections of cuttings.

No less important characteristic of woody plants cuttings regenerative ability is the number of adventitious roots formed on them (table 1).

Another picture concerning the correlation of the assessment of used drug effectiveness was formed by this attribute of rhizogenesis (table 1). Some of them caused an increase in the considered indicator, the highest average values were registered in the options of using heteroauxin (24.55±1.08 pcs.) and ferovit (22.00±1.38 pcs.), which is by 7.50 pcs. or 1.44 times and 4.95 pcs. or 1.29 times higher than that in the control, respectively. In addition, two other drugs showed results that exceeded the control: ecofus – 20.30±1.07 pcs. (by 3.25 pcs. or 1.19 times); epin – 18.65±0.90 pcs. (for 1.6 pcs. or 1.09 times) respectively. However, the use of a large number of reagents did not
cause a stimulating experience. On the contrary, there was a clear decrease in the number of neoplasms (in our case, adnexal roots), which was clearly manifested in the options with the use of profistim (9.00 ± 0.69 pcs.) and zircon (11.70±1.16 pcs.). The decrease in the scores compared to the control, respectively, was: -8.05 pcs. or 0.53 times and -5.35 pcs. or 0.69 times. The correlation of the control option (17.05±1.02 pcs.) with the size of the ‘Total’ option (16.67±0.39 pcs.) did not allow to clearly recognize that the use of the stimulating effect. The deviation in the smaller column was by 0.39 pcs. or 0.98 times.

A quiet contrast indicator of the success of the regeneration processes is the length of the leading root (table 1). This indicator of postregeneration development activity of root systems basically corresponded with the above mentioned assessments of callus and rhizogenesis (table 1) despite the fact that its unit had specific ratio values for different biostimulants (table 1). In this aspect, the most effective was Ecofys. Its estimates (27.88±0.65 cm) exceed the corresponding control value (23.01±0.69 cm) by 4.87 cm or 1.21 times. The average values of this parameter are quite high in the options of application of heteroauxin (24.06±0.52 cm) and kornevin (24.81±1.02 cm), which formed an excess over the control by 1.05 cm or 1.05 times and by 1.79 cm or 1.08 times, respectively. The minimum length of the leading root occurred in the options of the use of the preparations siliplant (19.40±0.56 cm) and of zircon (17.81±1.40 cm). They were inferior to the control by 3.62 cm and 5.20 cm, with a ratio of less than one: 0.84 times and 0.77 times. The generalized variant (22.93±0.31 cm) differed little from the control value (23.01±0.69 cm). In general, the noted differences in the effectiveness of the tested drugs indicate the need for an individual approach to their prescription.

Table 1. Indicators of callus formation, rhizogenesis and parameters of the aboveground part of cuttingsa,b.

| Preparations | Intensity of callus formation, % | Number of subordinate roots, pcs | Length of the leading root, cm | Total length of root systems, cm | Height of the aboveground part, cm | Diameter of the root neck, mm |
|--------------|---------------------------------|---------------------------------|------------------------------|---------------------------------|---------------------------------|-------------------------------|
| Heteroauxin  | 93.50±1.46                      | 24.55±1.08                      | 24.06±0.52                   | 266.92±16.71                    | 18.82±0.41                      | 3.70±0.08                     |
| Kornevin     | 89.00±2.63                      | 16.55±1.03                      | 24.81±1.02                   | 188.58±13.91                    | 17.44±0.43                      | 3.47±0.08                     |
| Profistim    | 98.50±1.05                      | 9.00±0.69                       | 22.39±1.16                   | 115.37±11.69                    | 16.84±0.45                      | 3.47±0.10                     |
| Siliplant    | 92.00±2.18                      | 13.20±0.83                      | 19.40±0.56                   | 137.37±10.35                    | 16.93±0.36                      | 3.32±0.09                     |
| Ferovite     | 94.00±1.78                      | 22.00±1.38                      | 23.15±0.85                   | 236.18±11.81                    | 16.52±0.27                      | 3.82±0.10                     |
| Zircon       | 43.00±4.82                      | 11.70±1.16                      | 17.81±1.40                   | 134.69±14.95                    | 16.05±0.43                      | 3.40±0.10                     |
| Ecofus       | 89.00±2.14                      | 20.30±1.07                      | 27.88±0.65                   | 272.22±14.42                    | 18.43±0.58                      | 3.63±0.07                     |
| Extrasol     | 96.50±0.76                      | 13.65±0.92                      | 23.79±1.05                   | 147.17±10.59                    | 17.01±0.43                      | 3.76±0.12                     |
| Epin         | 71.00±3.47                      | 18.65±0.90                      | 22.99±0.47                   | 191.81±10.72                    | 18.04±0.46                      | 3.89±0.06                     |
| Control      | 78.50±3.96                      | 17.05±1.02                      | 23.01±0.69                   | 222.92±16.22                    | 15.12±0.49                      | 3.25±0.10                     |
| Total        | 84.50±1.17                      | 16.67±0.39                      | 22.93±0.31                   | 191.32±4.97                     | 17.12±0.15                      | 3.57±0.03                     |

aThe number of primary sampling units in each option – 40; number of primary sampling units in a generalized array (option Total) for each indicator, the regeneration ability – 400.

bControl – water; Total – generalized value.

The characteristic of rhizogenesis which is traditionally esteemed to be the most informative is the total length of all the roots formed on each of the cuttings (table 1). In this case, generally there is a very limited list of incremental processes stimulators that promote the growth of adnexal roots (table 1). With the best positions, it includes heteroauxin (266.92±16.71 cm) and ecofus (272.22±14.42 cm). Their advantage over the control was 44.00 cm or 1.20 times and 49.30 cm or 1.22 times. Among all the drugs esteemed in this experiment only one, ferovit, was able to enhance the linear growth of roots. Noticeably inferior to the results of heteroauxin and ecofus use, it
nevertheless ensured that the root systems achieved a total length (236.92±11.81 cm), which exceeded the level of the control variant (222.92±16.22 cm) by 13.26 cm or 1.06 times. To the greatest extent in the development of root systems, cuttings of the option with profistim use lagged behind. Their result (115.37±11.69 cm) is by 107.55 cm or 1.93 times lower than the control and by 75.95 cm or 1.66 times lower than the average generalized for the entire experimental data base for this feature. In general, it is possible to state that the material obtained in this order is statistically significant and reliable, which is confirmed by the calculated values of the Student's t-criteria and the accuracy indicator of the experiment (relative error-P), which corresponded to the tabular limits at the five percent and one percent significance levels.

All the tested substances and the water used as a control caused the appearance of callus and the formation of adnexal roots in absolutely all the cuttings planted, for this reason their rootability was the same in all variants of the experiment and amounted to 100 %. The obtained result also indicates the absence of an inhibitory effect of the tested substances and indicates their zero toxicity for the studied species.

The significance of differences in the effectiveness of biologically active drugs use in cutting rooting was assessed by one way ANOVA (table 2).

| Attribute | \(F_{exp}\) | \(\text{h}^2\) \(\pm s_h^2\) | \(F_h^2\) | \(\text{h}^2\) \(\pm s_h^2\) | \(F_h^2\) | LSD\(_{05}\) | D\(_{05}\) |
|-----------|-------------|-----------------|-------------|-----------------|-------------|-------------|-------------|
| Attribute 1 | 38.16 | 0.468 \(\pm 0.012\) | 38.160 | 0.482 \(\pm 0.012\) | 40.257 | 7.435 | 12.258 |
| Attribute 2 | 22.50 | 0.342 \(\pm 0.015\) | 22.505 | 0.349 \(\pm 0.015\) | 23.297 | 2.795 | 4.609 |
| Attribute 3 | 9.77 | 0.184 \(\pm 0.019\) | 9.766 | 0.179 \(\pm 0.019\) | 9.497 | 2.420 | 3.989 |
| Attribute 4 | 4.76 | 0.099 \(\pm 0.021\) | 4.760 | 0.086 \(\pm 0.021\) | 4.074 | 1.013 | 1.671 |
| Attribute 5 | 18.20 | 0.296 \(\pm 0.016\) | 18.200 | 0.301 \(\pm 0.016\) | 18.632 | 36.390 | 59.995 |
| Attribute 6 | 6.24 | 0.126 \(\pm 0.020\) | 6.239 | 0.116 \(\pm 0.020\) | 5.677 | 1.347 | 2.221 |
| Attribute 7 | 8.36 | 0.162 \(\pm 0.019\) | 8.359 | 0.155 \(\pm 0.019\) | 7.972 | 0.190 | 0.313 |
| Attribute 8 | 18.18 | 0.296 \(\pm 0.016\) | 18.183 | 0.301 \(\pm 0.016\) | 18.615 | 0.338 | 0.558 |
| Attribute 9 | 17.24 | 0.285 \(\pm 0.017\) | 17.244 | 0.289 \(\pm 0.016\) | 17.597 | 0.735 | 1.212 |
| Attribute 10 | 6.48 | 0.130 \(\pm 0.020\) | 6.476 | 0.120 \(\pm 0.020\) | 5.932 | 1.197 | 1.974 |
| Attribute 11 | 5.54 | 0.113 \(\pm 0.021\) | 5.540 | 0.102 \(\pm 0.021\) | 4.918 | 0.254 | 0.418 |
| Attribute 12 | 3.12 | 0.067 \(\pm 0.022\) | 3.122 | 0.050 \(\pm 0.022\) | 2.299 | 3.920 | 6.464 |

\(^a\)Indicators: \(F_{exp}\) – experienced Fisher (\(F_{05/01}=1.90/2.41\)); \(\text{h}^2\) – is the proportion of the influence of organized factor; \(\pm s_h^2\) – error of the proportion of the influence of organized factor; \(F_h^2\) – confidence rating of influence; LSD\(_{05}\) – least significant difference; D\(_{05}\) – Tukey test.

\(^b\)Attributes: 1) callusogenesis, %; 2) the number of adventitious roots; 3) length of leading root; 4) the minimum length of adventitious roots; 5) the total length of adventitious roots; 6) the average length of adventitious root; 7) the indicator of balance in the development of root systems; 8) the ratio of the height of the aerial part of the stem to the number of adventitious roots; 9) the ratio of the diameter of the root neck to the number of adventitious roots; 10) the height of the aerial parts; 11) the diameter of the root neck; 12) the ratio of the height of the aerial part to the diameter of the root neck.

\(^c\)The number of primary sampling units for each of the features in the one-factor variance complex is 400. The total volume of the database in the analysis is 4800 units.

For all indicators of the regenerative ability of cuttings (callus and rhizogenesis), the fact of significant differences was confirmed by the Fisher criteria, the calculated values of which exceeded the permissible table values at both the 5% and 1% levels of significance. According to these characteristics, the experimental values of the F-test took values from 4.76 (Attribute 4 – the minimum length of adventitious roots), to 38.16 (Attribute 1 – the intensity of callus formation), which is significantly higher than the established statistical limits (\(F_{05/01}=1.90/2.41\)). According to the characteristics of the aboveground part of the rooted cuttings (Attribute 10–12), the Fischer criteria, in
general, are smaller, but also exceed the permissible table limits, taking values of 3.12–6.48. This allowed us to reject the null hypothesis about ANOVA’s no difference between the compared options for the use of stimulating the processing of biologically active substances and move to evaluate the effect of organized factor experience. In the calculations according to the Plohinsky algorithm, the share of its influence (in our case, the influence of differences between the stimulators themselves) on the formation of the general background of phenotypic variability for different signs is not the same. In particular, according to the intensity of callusogenesis (Attribute 1) and the total number of subordinate roots formed (Attribute 2), it is quite large, and quite significant: 46.8±1.2% (F_{p}^2=38.16) and 34.2±1.5% (F_{p}^2=22.50), respectively. This is the highest evaluation in the present dispersion of the complex.

The influence of differences in the used preparations on the resulting effect of dispersion in some cases was similar: in the total length of the adventive roots (Attribute 5) – 29.6±1.6% (F_{p}^2=18.20); in the ratio of the height of the aboveground part of the cuttings to the number of adventive roots(Attribute 8) – 29.6±1.6% (F_{p}^2=18.18); in the ratio of the diameter of the root neck to the number of adventive roots(Attribute 9) – 28.5±1.6% (F_{p}^2=17.24). The calculations with use of the Snedekor algorithm gave quite a comparable result (table 2).

The residual dispersion, the presence of which is traditionally considered as a manifestation of the non-directional influence of external conditions (unorganized factor), was also very noticeable and prevailed in all options of the experiment: from 53.17% (Attribute 1 – activity of callus formation) to 93.28% (Attribute 12 – the ratio of the height of the aboveground part to the diameter of the root neck). Criteria for the significance of differences (LSD_{0.05} and D_{0.05}) determined the level of arithmetic difference in the average values of a particular characteristic. It the level is broken the differences between the compared options (used stimulants) will be found to be significant, particularly if its value will be achieved 5% value. As for the activity of callusogenesis (Attribute 1), the actual difference in the mean (see table 1) has exceeded the level of LSD_{0.05} in all the options for the use of stimulants (9 from 9 cases) and in only 6 cases from 9 – when Tukey D-test was used (table 2). As for the number of formed adventitious roots (Attribute 2), after the pairwise comparison of the stimulants use characteristic option average values with control another result was observed: the actual difference in the mean (see table 1) exceeded LSD_{0.05} only in several options of stimulants use (7 from 9 cases), and only in 4 cases from 9 – when Tukey D-test was used (table 2).

The differences while using different options of stimulants at the background of other attributes by the parameters of the aerial parts of their rooted cuttings manifested weakly. First of all it may be explained by the equalitarian approach to the formation of the samples for filling their options. Initially, the length and diameter of the cuttings were equalized and the possibility of intentionally introducing any longer or larger cuttings into any experiment options was excluded in principle. Eventually it was established by the analysis of variance. At the same time, the post-regenerative development of the aboveground part, both in height and diameter, could not help suffering an influence of inequality in the formation of callus, in the appearance of adventitious roots and their linear growth, which were caused by the unequal stimulating effect of the tested drugs. The consequence of this influence is the differences between the processing options, which have reached the level of significant ones, despite the fact that here the share of the influence of the organized factor on the formation of differences is much smaller. Thus, callusogenesis and rhizogenesis were a kind of test factor that ensured the differentiation of the compared groups of cuttings according to their processing options. The obtained result is explained by the different biological activity of the preparations used in relation to common privet and, as a result, their different effectiveness as stimulators of the regeneration processes of the studied species.

4. Conclusion
The tested stimulants were not equally active in their influence on the course of various processes of regeneration and post-regenerative development of root systems and the aboveground part of cuttings. The best effect in stimulating callus formation was observed in profistim (98.50±1.05%) and extrasol...
(96.50±0.76%). The greatest total length of root systems was formed under the influence of ecofusc and heteroauxin. The maximum number of subordinate roots arose when using heteroauxin and ferovite. The advantages in the linear growth of the aboveground part were demonstrated by cuttings treated with heteroauxin (18.82±0.41 cm) and ecofusc (18.43±0.58 cm). At the same time, cuttings treated with ferovite (3.82±0.10 mm) and epin (3.89±0.06 mm) had the largest diameter of the root neck. Heteroauxin and ecofusc are classified as the most effective stimulants according to the largest number of important indicators of the regenerative ability of cuttings of privet common.

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