

Improvement of grafting procedures for the ornamental species: I. *Picea pungens* Engelm. var. *glauca* Regel

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Abstract. In order to get ornamental trees for landscaping, Colorado blue spruce (*Picea pungens* Engelm. var. *glauca* Regel) scions were grafted on Norway spruce (*Picea abies* (L.) Karst.) rootstocks. An original double-side-veneer grafting method was applied in five experimental or basic variants. The plastic tapes and the ecological Ceraltin[®] wax developed by the Research and Development of Bio-stimulators (CCDB) BIOS Cluj were tested. In addition, two controls in which the classic raffia and the traditional hot wax and the classic side-veneer-grafting method were used. The obtained results, expressed in percents, were transformed in arcsin square root of percent values, and then a two-way analysis of variance (ANOVA) was used. Highly significant ($p < 0.001$) statistical differences were found between all grafting variants, including controls. The Duncan test proved that original double-side-veneer grafting method and the new developed materials, i.e. plastic tapes and the ecological Ceraltin[®] wax have contributed in getting a better grafting success compared to the controls. Thus, the double-side-veneer grafting method and the two grafting materials are highly recommended to be used in getting grafted Colorado blue spruce ornamental trees.

Keywords *Picea pungens* var. *glauca*, *Picea abies*, side-veneer-grafting, double-side-veneer grafting, Ceraltin[®] wax, plastic tapes, raffia, analysis of variance, Duncan Test.

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Introduction

The origin of grafting can be traced ancient times. Bailey (1891) described and illustrated

the methods of grafting and budding commonly used in the United States and Europe at that time. The methods used today differ very little from those described by Bailey (Hartmann &

Kester 2002). Similarly, **Bouvarel (1960)** have mentioned that grafting has been used from the earliest times and it is still used on a large scale to preserve and multiply desired genotypes. Grafting is a basic tool for horticulturists and also has been used widely in forestry for clone preservation and seed orchard establishment (Zobel & Talbert 1984). **In forestry, the grafting** was used almost exclusively for the clonal propagation of genetically improved seed orchards in many species, such as Monterey Pine (*Pinus radiata* D. Don), Hoop Pine (*Araucaria cunninghamii* Ait.), Slash Pine (*Pinus elliottii* Engelm. var. *elliottii*), Caribbean Pine (*Pinus caribaea* Mor.), Eucalyptus (*Eucalyptus* sp.) Douglas Fir (*Pseudotsuga menziesii* (Mirb.) Franco, and many others (Porada 1993). The *veneer-grafts* on established rootstocks are most common in Douglas-Fir (Radu & Blada 1966, Silen & Copes 1972).

Methods of grafting are numerous and they were covered in many texts, among others Bailey (1891), **Dormling 1964**, **Hartmann & Kester (1983)**, **Dorman (1976)**, **Garner (1979)**, **Hartmann & Kester (2002)**, **Stănică et al. (2002)**, **Smith (2007)**. Some species, including oaks, do not graft easily, and adjustments to the usual methods must be made to obtain a reasonable degree of success (Hatmaker & Taft 1966). Often, it is not pure grafting technique that results in failure, but rather incorrect grafting operations and the poor care given to the scion or rootstock before, during and after grafting or in grafted plants release (Zobel & Talbert 1984).

At conifers, **the side-veneer-grafting method** is the most frequently applied (**Teuscher 1962**, **Leiss 1987**, **Enescu, et al. 1994**, **Carpenter 1998**, **Lupescu 2003**, **Smith 2007**). With *P. pungens*, this grafting method is practiced in greenhouse, during August-September or December-March periods (**Hartmann & Kester 2002**, **Iliescu 2002**). The veneer grafting was recommended in alder, aspen, oak, Douglas-fir, larches, pines and spruces (Wright 1976)

Among other species, the spruce grafting

was included in the breeding programme of the Forest Research Institute (ICAS) Bucharest, which was implemented between 1960-1988 years. Within this period, the Norway Spruce grafting success have varied between 3 and 40% in greenhouse and between 0 and 21% in nursery (Blada, unpublished data). Almost similar success results (30 to 40%) in Norway spruce were reported by Enescu (1967). Compared to the present obtained results, the above reported ones were not satisfactory. The lack of know how explained by difficult access to the foreign literature, was the major reason of such poor results. Also, the primitive facilities and the classic grafting materials, such as raffia and **traditional hot wax significantly** contributed to the **unsatisfactory results**.

Recently, production of conifer ornamental trees has become a highly profitable business, not only for horticulturists but for foresters, as well. The current aim is to increase the grafting rate of success. For this, the grafter has to use efficiently modern grafting materials rather than the traditional ones.

The purpose of this experiment was to test: (i) the original double-side-veneer-grafting method as described below (Blada 2009), in comparison with the traditional side-veneer-grafting (Hartmann & Kester 2002), (ii) the performance of both plastic tapes and ecological Ceraltin® wax developed by CCDB-BIOS Cluj-Napoca which appeared clearly superior (Blada 2009), in comparison to the traditional side-veneer-grafting method and traditional materials like raffia and hot wax, (iii) which is the optimal grafting period, inside a non-climatized greenhouse i. e. early spring or late autumn grafting.

Materials and methods

Rootstocks

During the month of March 2009, the rootstock seedlings were potted in plastic bags of 22 cm

height and 18 cm in diameter. Prior to potting, the rootstocks were 27-35 cm in height, of 10-12 mm in root collar diameter and 4-7 mm diameter of stem's thickness at the grafting area.

In the nursery, the potted rootstocks were placed in a bed of 1 m wide and 22 cm depth. In order to maintain the humidity inside the bed, a five to six cm thickness layer of sawdust was placed among the potted seedlings. During the summer time, to the rootstock plants, the specific care as practiced in forestry nursery was applied. In such conditions, the rootstocks have reached normal suitable size for grafting, i.e. the rootstocks were the same or slightly larger in diameter than the scions (Figure 1). At the end of October 2009, the pre-potted rootstocks were transferred in a heated greenhouse belonging to the Cormaia-Anies Forest District (Bistrița County).

Scions

Three mature blue spruce trees, of unknown origin, with blue-silver color foliage were the source of the fresh scions which were collected a day prior to grafting, so that it was not necessary to keep them in the fridge. In order to have suitable size (4-5 cm length, 3-7 mm thickness), the scions were collected from the upper part of the crown. It should be taken into account that the grafting success in silver spruce and in any other species as well, is higher if the period between scions collection and their grafting is shorter.

Tying and waxing materials

The grafting materials tested by this experiment were the plastic tape and the ecological Ceraltin® wax. The following grafting tapes were applied on the joined rootstock - scion:



Figure 1 Sample of silver spruce rootstocks prepared for grafting (original photo: I. Blada)

(i) the blue color plastic tape of 0.11 mm thickness and 13 mm width; since this tape has a remarkable plasticity, in some cases, the application of grafting wax may be eliminated, (ii) the yellow color plastic tape of 0.12 mm thickness and 13 mm width; has a lower plasticity compared to the blue color tape. These tying materials can seal and help maintain a high relative humidity in the graft union area.

The Ceraltin® grafting wax plays multiple roles: (i) it seals over the graft union thereby preventing the loss of moisture and death of the tender, exposed cells of the cut surface of the scion and rootstock; the health of these cells is essential for callus production and healing of the graft union, (ii) it stimulates the callus production, (iii) it prevents the entrance of various decay-producing organisms that rot wood, (iv) it prevents the air and water to enter from outside between the two grafted partners, thus avoiding the oxidizing of cell juice at the union level area.

Both the plastic tapes and the Ceraltin® grafting wax were applied only to the basic grafting variants (V.0 to V.4) while the traditional raffia and hot wax were used for tying and waxing, respectively, the two control variants (V.5 and V.6). The raffia consists of strips of palm leafstalk fibre which is an older wrapping material but still used (Hartmann & Kester 2002). The used classic wax was in accordance with that recommended by Enescu (1967).

Grafting operation

Two grafting methods were used in the present experiment i.e. the **side-veneer-grafting** (Hartmann & Kester 2002), applied to the two control variants and the original **double-side-veneer-grafting** (Blada 2009), which were applied to the five experimental variants. The **side-veneer-grafting method** has proven useful for large scale propagation of nursery trees (Leiss 1987) and for grafting small potted plants including conifer seedlings (Hartmann & Kester 2002, Smith 2007).

For the **side-veneer-grafting** illustrated in the Figures 2abc the following details are given: (i) a **shallow downward and inward cut** (like a tongue) from 25 to 38 mm long is made in a smooth area in the crown of the rootstock; at the base of this cut (tongue), a second short inward and downward cut is made, intersecting the first cut (tongue), so as to remove the piece of wood and bark, i.e. the side tongue (Figure 2a, **left side**), (ii) **the scion is prepared** with a long cut along one side and a very short cut at the base of the scion on the opposite side (Figure 2a, **right side**), **these scion cuts should be the same length and width as those made in the rootstock**, so that the vascular cambium layers can be matched as closely as possible. Then the scion is ready to be **inserted into the rootstock cut** (Figure 2b). After inserting the scion into the rootstock cut, both components are tightly wrapped with plastic tape (Figure 2c) then the waxing follows, but it was not applied, yet.

Generally, **double-side-veneer-grafting method is more or less similar to the side-veneer-grafting one**, but it possess some essential specific characteristics, i.e. it creates the possibility of callus developing on both sides of the rootstock and scion. Technical characteristics of this method are illustrated in the Figures 3abcd, with the following details: (i'') the scion is prepared with a 4 to 5 cm long cut along both sides of the scion (Figure 3a), **these two scion cuts should be the same length and width as those made in the rootstock** so that the vascular cambium layers can be matched as closely as possible on both sides, (ii'') the shallow downward and inward cut from 4 to 5 cm long in the crown of the rootstock but the piece of wood and bark which resulted, i.e. the side tongue, was not removed (Figure 3b), by keeping this tongue on the place, **four contact cambium layers** (two on rootstock and two on scion) will be involved in callus formation; with side-veneer-grafting only two contact layers contribute to the callus formation. Then the scion was inserted into the rootstock cut

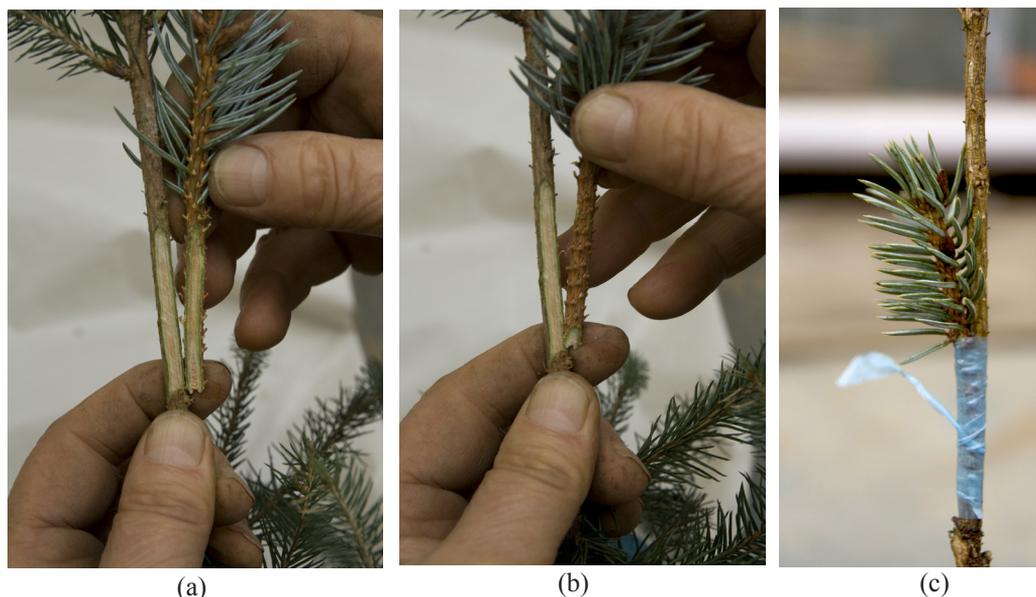


Figure 2 Illustration of the side-veneer grafting: (a) the long cuts made on both rootstock (left side) and scion (right side); (b) the scion is ready to be inserted into the rootstock cut; (c) after inserting the scion, the graft is tightly tied with plastic tape (original photo I. Blada)

(Figure 3c) and finally, the two grafting components were tied together (Figure 3d).

The timing of grafting is variable, according to the species and the available facilities. In Oregon, optimum periods for spruce species grafting in the greenhouse are July-August and December-January-February (Meacham 1995). In this study, the grafting was accomplished in two time periods: the first period took place in November of the 2009, when the buds of the rootstocks were not flushed, while the second period took place on March 2010, on rootstock whose buds were flushed. The double-side-veneer grafting was applied to the five (V.0, V.1, V.2, V.3, V.4) experimental or basic variants while the side-veneer grafting was applied only to the two controls (V.5 and V.6) variants (Table 1).

Therefore, the experimental grafting design structure consisted of seven variants x four replications x 20 seedling per replication, i. e. a total of 560 grafted seedlings on the whole ex-

periment. Of the seven variants, five (V.0, V.1, V.2, V.3, V.4) were experimental or basic and the other two (V.5, V.6) were controls. Some grafting variants were illustrated in the Figures 4 abcd.

According to their grafting variant, immediately after grafting termination, all grafted seedlings were labelled.

Greenhouse microclimatic

Certain environmental requirements must be met for callus tissue to develop. Therefore for a successful grafting, inside the greenhouse had to be maintained some specific microclimatic condition. For general callusing purposes, temperatures from 20 to 25°C and 85 to 95% atmospheric humidity was the most satisfactory. However, because the greenhouse was more or less a primitive one, during the months subsequently the grafting, especially in the days with insolation, the temperature has



Figure 3 Illustration of the double-side-veneer grafting: (a) the scion was prepared with 4-5 cm long cut on both sides; (b) the rootstock cut preparation; (c) the scion was inserted into the rootstock cut; (d) the grafting partners are tightly tying with plastic tape then the Ceraltin[®] wax will be applied (original photo I. Blada).

exceeded the above mentioned values; consequently, some losses in grafted plants have occurred. In such days, artificial misting was done inside the greenhouse.

Fine watering was accomplished four times per day. Special care was taken at the soil moisture from plastic pots in which the plants were growing. It is well known that an exce-

ssive moisture from pots, may cause the root rot; to avoid such phenomenon, it was applied the principle that the soil should be moist, but not wet.

Lopping the rootstocks

After graft union formation has occurred, it

was necessary to force out the scion or the scion bud. To give power to the scion, just before grafting some branches from the rootstock were removed. Then, during the March-June, this operation was four times repeated. This helped to force out the scion and maintained growth of the grafted seedling. On late July when the graft union is accomplished and the

scion leader became the dominant shoot system, the rootstock top was cut off above the scion bud union; owing to this the scion leader rapidly elongated.

Aftercare of scion

As the silver spruce scions were collected from

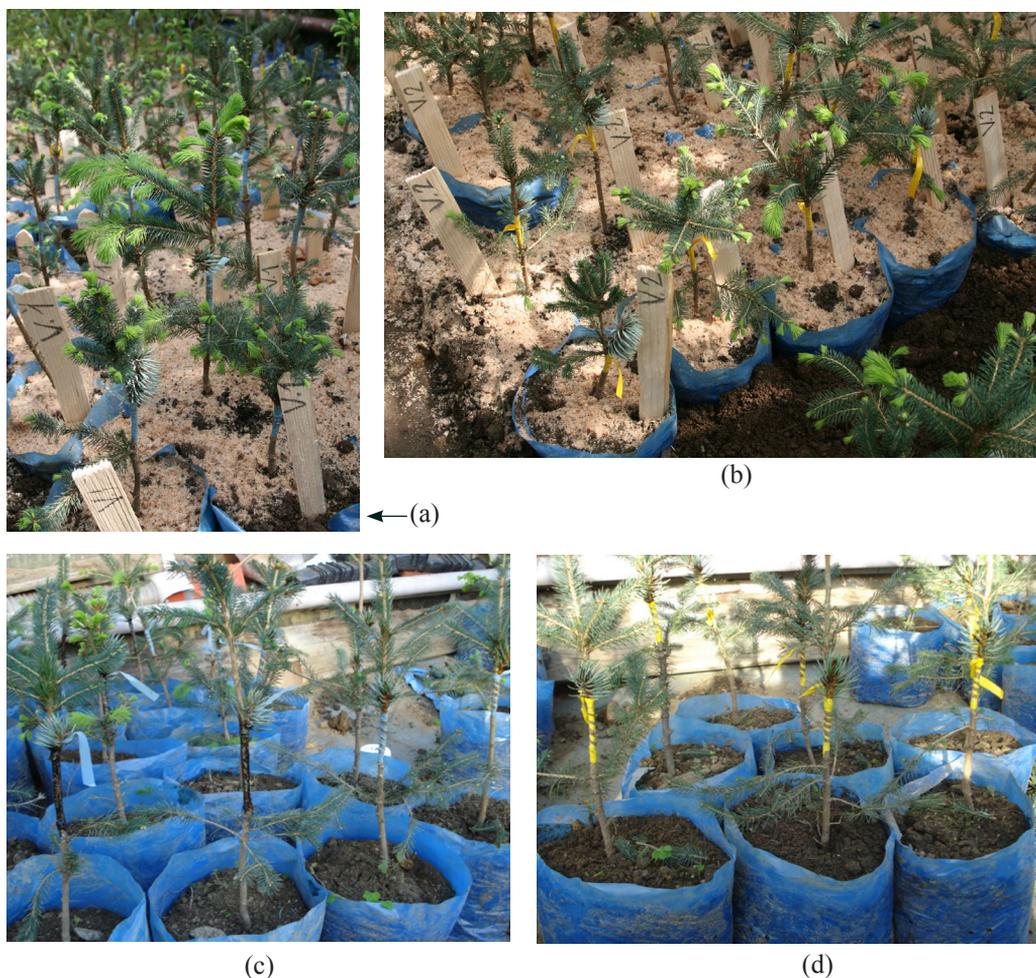


Figure 4 Samples illustration of the four basic grafting variants of the double-side-veneer grafting method: the variants (a) and (b) were both tied strip-near-strip with blue (V.1) and yellow (V.2) colour plastic tape, respectively; while the variants (c) and (d) were both tied strip-space-strip with blue (V.3) and yellow (V.4) colour plastic tape, respectively. The V.3 was partially waxed with Ceraltin® wax while the V.1, V.2 and V.4 were not, yet.

Table 1 Grafting variants and their distribution on the two grafting periods

Variant	Description
V.0	<p>First grafting period: November 12-14, 2009</p> <p>Tied strip-near-strip with blue colour plastic tape without Ceraltin® wax application.</p> <p>Second grafting period: March 28-31, 2010</p>
V.1	Tied strip-near-strip with blue colour plastic tape, without Ceraltin® wax application
V.2	Tied strip-near-strip with yellow colour plastic tape without Ceraltin® wax application
V.3	Tied strip-space-strip with blue colour plastic tape with Ceraltin® wax application
V.4	Tied strip-space-strip with yellow colour plastic tape with Ceraltin® wax application
V.5	Tied strip-near-strip with classical natural raffia with classical wax application (Control 1)
V.6	Tied strip-near-strip with classical natural raffia without classical wax application (Control 2)

the top of the tree, many female flower buds were ready to burst (flush). In order to prevent the grafting failure, the respective buds were removed prior to their opening; the remaining wounds were waxed with Ceraltin® grafting wax. To prevent girdling, the plastic tape was cut after the graft union completed.

Protection of new plants

Prior to transferring successfully grafted plants to the nursery, to each one a stick was planted into the pot soil near the seedling then the scion leader was tied to that stick. By this way, the scion breaking during the further plant handling was avoided.

Plant transfer outside greenhouse

On early July, the grafted seedlings were planted out in the experimental nursery where they were arranged according to their grafting variants. In order to avoid the losses, along the summer time, they have benefited of special care, such as shade, watering, digging, weeds and insects control.

Plant inventory and statistical analysis

On June 30, 2010, the successfully grafted plants were recorded separately on variants and replications, and the results expressed in

percents were transformed in arcsin $\sqrt{\%}$ values. These data were statistically analyzed by using a two ways analysis of variance (Ceapoiu 1968). Two variance analyses were performed: the first analysis has included all the seven variants, i.e. five basic and two controls while the second analysis included only the five basic variants. Finally, the Fischer's & Yates (1963) and Duncan (1955) tests were applied.

Results

The variance analysis shows that highly significant ($p < 0.001$) differences were found between the seven grafting variants (controls included) (Table 2, row 2, column 5). Similarly, the analysis which took into account only the five basic variants (controls excluded) clearly demonstrates the existence of highly significant ($p < 0.001$) differences between them (Table 2, row 5, column 5). The results of previously mentioned variance analyses suggested the possibility of selection of some grafting variants with a higher production potential compared not only with the two controls but also with some basic variants, themselves.

The ranking of, and the differences between variants according to the Duncan Multiple Range Test are given in Table 3, with the following details. (i) The V.0 variant which was grafted on mid-November, on non-flushed

rootstocks was placed at the top of the ranking with an average grafting success of 87%. (ii) The V.3 and V.4 variants whose scion and rootstock were tied strip-space-strip with plastic tape and waxed with Ceraltin® wax have occupied the 2nd and the 3rd places; they were grafted on flushed rootstock and have reached an average grafting success of 81 and 74%, respectively. (iii) The V.1 and V.2 variants have occupied the 4th and the 5th places in the rank; they were grafted similarly to the V.3 and V.4 variants with the exception that a tied strip-near-strip was applied; their grafting success was almost similar, i.e. 71 and 68%, respectively. (iv) As expected, the two controls (V.5 and V.6) have occupied the last places in the rank; their grafting partners were tied strip-near-strip with raffia and waxed with classic wax; their grafting success was 46 and 41%, respectively; therefore, the double-side-grafting method worked out for the silver spruce, where grafting success has climbed from mediocre 41% (control) to more than double, i.e. 87%. (v) Between the V.0 variant whose

grafting took place on mid-November, on non-flushed rootstock, on one hand, and the V.3, V.4, V.1, V.2, V.5 and V.6 variants where the grafting took place, on March, on flushed rootstocks there were found highly significant ($p < 0.01$) differences whose magnitudes were as much as 6%, 13%, 16%, 19%, 41% and 46%, respectively (Table 3, row 1, columns 3 to 8). (vi) Between the V.3 variant, on one hand, and the variants V.4, V.1, V.2, V.5 and V.6, on the other hand, where the grafting took place on flushed rootstock, there were found significant ($p < 0.05$) and highly significant ($p < 0.01$) differences whose magnitudes were 7%, 10%, 13%, 35% and 40% respectively (Table 3, row 2, columns 4 to 8). (vii) Between the V.4 variant, on one hand, and the variants V.1, V.2, V.5 and V.6, on the other hand, there were found significant ($p < 0.05$) and highly significant ($p < 0.01$) differences whose magnitudes were of 3%, 6%, 28% and 33%, respectively (Table 3, row 3, columns 5 to 8). (viii) Between the variant V.1, on one hand, and variants V.2, V.5 and V.6, on the other hand, significant ($p < 0.05$)

Table 2 Analysis of variance

Row	Source of variation	SSD	D.f	MS	F
ANOVA for basic plus control variants					
1	Replications	1.70	3	0.57	0.44
2	Variants	2731.10	6	455.18	349.65***
2	Error	23.43	18	1.30	
ANOVA for basic variants, only					
4	Replications	0.95	3	0.32	0.20
5	Variants	470.06	4	117.52	74.91***
6	Error	18.83	12	1.57	

Table 3 Differences (%) between grafting variants, Duncan’s Multiple Range Test

Row	Variants	Mean (%)	V.3 81	V.4 74	V.1 71	V.2 68	V.5 46	V.6 41
1	V.0	87	6**	13**	16**	19**	41**	46**
2	V.3	81		7**	10**	13**	35**	40**
3	V.4	74			3*	6**	28**	33**
4	V.1	71				3*	25**	30**
5	V.2	68					22**	27**
6	V.5	46						5**
7	V.6	41						0

Note: * - $p < 0.05$, ** - $p < 0.01$

and highly significant ($p < 0.01$) differences were recorded (Table 3, row 4, columns 6 to 8). (ix) No statistically significant differences were found between the variant V.2 and any other basic variant; instead highly significant ($p < 0.01$) differences were found between it and the two controls (Table 3, row 5, columns 7 and 8). (x) All basic variants, i.e. V.0, V.3, V.4, V.1 and V.2 were highly significantly ($p < 0.01$) different of the two controls (Table 3, rows 1 to 5, columns 7 and 8).

Discussion

The spruce grafting was included in the breeding programme of the Forest Research Institute of Bucharest. During its implementation across 30 years, the spruce grafting success has varied between 3 and 40% in greenhouse and between 0 and 21% in nursery. Because the ornamental trees, including blue spruce, are in high demand, the above mentioned grafting results are not satisfactory. That is why the modern plastic tape and the ecological Ceraltin[®] wax including the original double-side-veneer grafting method were tested in the present experiment. In addition, the grafting operation was applied not only in the spring (March) on flushed rootstocks but also in late autumn (November) on non-flushed rootstocks. Consequently, the newly acquired results in this study have to answer to the following questions: (i) in a non-climatized greenhouse, is the November time grafting more adequate than the March one? (ii) are the new developed plastic tape and Ceraltin[®] wax more efficient than the classics raffia and hot wax, respectively? (iii) is the new double-side-veneer grafting method more efficient than the classic side-veneer grafting?

Concerning the 1st question, it was started from the hypothesis according to which the temperature should be maintained below 25°C and the air humidity between 80-90%. In the present non-climatized greenhouse the pre-

viously mentioned climate parameters could be maintained only during the cool period of the year so that they have favoured the callus formation, i.e. the grafting success. This is the explanation according to which the success of late autumn grafting for the variant V.0 reached the value of 87% (Table 3, row 1, column 2), although it did not benefit of the stimulation effect of the Ceraltin[®] wax. Therefore, it is better to accomplish the grafting during the month of November than in March.

Concerning the 2nd question, definitely the new developed plastic tape and Ceraltin[®] wax were more efficient than the classics raffia and hot wax, respectively. For example, the 87% grafting success with the V.0 variant, where the blue colour plastic tape was tied strip-near-strip without Ceraltin[®] wax application is good evidence in comparison with the V.5 control variant. Similarly, the V.3 variant with 81% grafting success (Ceraltin[®] wax application) proved its ability in callus formation owing to which reached higher success compared to the control.

The grafting success with all basic experimental variants at which the double-side-veneer grafting method was used differed highly significantly of the two control variants; therefore, the double-side-veneer grafting method was much better than the traditional side-veneer grafting one. For example, the V.0 grafting variant has reached 87% grafting success which means a superiority of 117.5% compared to the 40% success reported by Enescu (1967).

Conclusion

The in November grafting on non-flushed rootstocks, in heated greenhouse, by tying strip-near-strip of blue colour plastic tape and without Ceraltin[®] wax application represents a premiere with woody species, for Romania. The obtained better results with the November grafting suggest that the March grafting could

be replaced by the former one; by this way, the non-climatized greenhouses could profitably be used.

As the plastic tape and Ceraltin® wax proved to be more efficient than the classic ones, it is recommendable to discard the latter and to promote the former ones. Consequently, for a better efficiency in blue spruce propagation, all basic or experimental grafting variants should be used; however, the priority should be given to November grafting and those of March ones which employed the Ceraltin® wax applied on strip-space-strip tied tape.

In terms of grafting success, it can be said that the double-side-veneer grafting method is superior to the classic side-veneer-grafting one; therefore the former method should be used on large-scale for the production of blue spruce grafted ornamentals.

At the same time, additional improvements of the success rate may result from detailing green house conditions, temperature control, frequency and amount of misting, quality of wax used and other factors.

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