DENDROBIOLOGY
2002, vol. 47, Supplement: 93–100

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Die-back of grafts in the clonal seed orchards of Douglas-fir (*Pseudotsuga menziensii* [Mirb.] Franco) in Poland and attempts at producing seedlings of this species by autovegetative propagation

Abstract: So far, experience in the existing clonal seed orchards of Douglas-fir indicates that grafts of this species are short-lived. According to research carried out in North America, incompatibility between the grafted scions and the rootstocks happens during the whole period of growth and development in clonal seed orchards of Douglas-fir. A similar phenomenon occurs in the clonal seed orchards of Douglas-fir in Poland. The extent of die-back of grafts was examined in three existing oldest units in the Forest District of Gniewkowo (founded in 1992–1993; 2.80 ha), the Forest District of Leżajsk (founded in 1995, 4.22 ha) and the Forest District of Łopuchówko (founded in 1993, 7.43 ha). In all these areas, losses due to die-back ranged from a dozen to a few dozen per cent. Die-backs in the clonal seed orchards in the Forest District of Gniewkowo and in the Forest District of Łopuchówko are now reduced to as much as about fourty per cent of grafts. Analyses of die-back in all these areas reveal great variation between individual clones, indicating its genetic basis. Research carried out so far has not demonstrated the presence of any pathogens responsible for causing the die-back in the grafts. Some hope to solve the problem mentioned above arises from attempts at autovegetative propagation of Douglas-fir.

Key words: grafting, vegetative propagation, rooting

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Introduction

So far, experiments conducted in clonal seed orchards of Douglas-fir indicate that grafts of this species are short-lived plants. According to research carried out in North America, incompatibility in grafts of Douglas-fir between scion and rootstock happens during the whole period of growth and development in clonal seed orchards. That phenomenon is observed also in clonal seed orchards of Douglas-fir in Poland. Here scion-rootstock incompatibility often develops to the greatest extent after 8–12 years of growth. An eight-year-old clonal seed orchard in Belgium where 50 per cent of grafts died back as well as a ten-year-old one in Germany where, due to the grafts die-back, only 30 per cent of the grafts remained can serve as examples (Mejnartowicz 1996).

In the USA there has been tested a method of improving translocation (flow) between the rootstock and the scion by making a crosswise bark incision at the place where those parts of grafts are joined. As it has been shown, this intervention can hugely increase seedling viability. That conclusion has been re-enforced by the evaluation of the annual index of the die-back of the grafts before and after the operation (Copes 1989).

In Italy, there have been revealed differences in survival of grafts depending on the season of grafting. It has been found that seedlings grafted in spring are more viable in contrast to those grafted in summer (Giannini et al. 1988).

In spite of the research conducted so far, the problem of the die-back of grafts in Douglas-fir seed orchards is still serious and is causing losses in already existing units. In Poland, the die-back is observed in all units set up before 1996.

The grafts die-back inventory of the clonal seed orchards of Douglas-fir in Poland established before 1996

The examined clonal seed orchards

The author of this paper has conducted research in three clonal seed orchards of Douglas-fir established in Poland before 1996:

1. The clonal seed orchard in the Forest District of Gniewkowo, in the Forest Area Wielowieś, planted in 1992–93; area: 2.80 ha, number of clones used: 37; number of out-planted grafts: 694.
2. The clonal seed orchard in the Forest District of Leżajsk, in the Forest Area Julin; planted in 1995; area: 4.22 ha; number of clones used: 73; number of out-planted grafts: 1038.
3. The clonal seed orchard in the Forest District of Łopuchówko, in the Forest Area Wojnowo; planted in 1993; area: 7.33 ha; number of clones used: 47; number of out-planted grafts: 2036.

Symptoms and alleged causes of the die-back

In all units the die-back of grafts proceeds gradually, starting from green needles turning yellow, later turning brown and dropping (Fig. 1). In the first stage of die-back, bleeding on the outer bark in the place of grafting often takes place. During the growth of grafts diameter disproportion between the rootstock and the scion often arises. Subsequently, a characteristic swelling on the shoot appears (Fig. 2). Frequently promisingly healthy grafts die back a few years after planting.

Fig. 1. The dried graft in the clonal seed orchard in the Forest District of Gniewkowo

Fig. 2. Swelling and graft bleeding in the grafting place on the died-back graft

Grafts are also prone to break away in the place of grafting. Such situations are often caused by especially strong winds. Then vivid green plants, not having shown any symptoms of die-back, very often break (Fig. 3). On observing the place of transition between the rootstock and the scion, knitting of tissues in the area of wood and their big antagonism in the area of cambium and phloem can be detected. There are also situations where weak joints of tissues are visible on the whole cross section of the grafting place (Fig. 4).

To try and identify why the problem of grafts breaking away occurs in the existing clonal seed orchard in the Forest District of Gniewkowo, fragments of the grafts that died back have been sent to the Forest Protection Department in Gdańsk for examination.

Samples of the material have also been sent to the Phytopatology Department of the Academy of Agriculture in Cracow. In both institutions tests have been conducted to isolate substances accompanying necroses.

The results of the research have definitely excluded fungous pathogens as possible factors causing symptoms observed in the clonal seed orchards. It seems that abiotic factors are the cause of the die-back.

The results of the grafts failure inventory and their interpretation

The present number of still viable grafts reduced by die-back in the examined clonal seed orchards is: Gniewkowo – 40%, Leżajsk – 11%, Łopuchówko – 40%.

The reduction of the number of grafts in the clonal seed orchards comes to a few to a dozen or so per cent within a year. In the clonal seed orchards in the Forest District of Gniewkowo that index has reached 1,3 % for the whole area, concerning only the losses appeared within the winter 2000/2001 (Fig. 5).

In the clonal seed orchard mentioned above there is a considerable variation between individual plots as far as dying of the grafts is concerned. It took place extensively during last winter when the largest losses were observed in the plot 1. It seems that the effect of location plays an important role in the die-back process in this area.

The influence of location occurs also to a greater extent in the clonal seed orchard in the Forest District

Fig. 3. The graft broken away by wind in the clonal seed orchard in the Forest District of Gniewkowo

Fig. 4. The cross shoot section in the place of breaking away of the scion

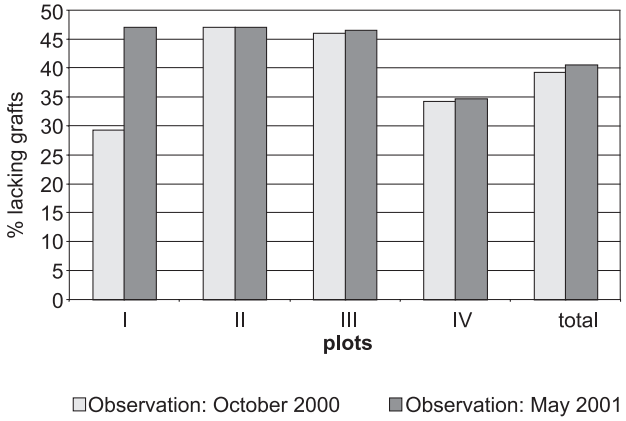

Fig. 5. Die-back of grafts in the nine-year-old clonal seed orchard in the Forest District of Gniewkowo in winter 2000/2001

of Leżajsk (Fig. 6) where up to 11 % of the grafts died back within 15 months (April 1998 – July 1999). Differences in the die-back index between individual plots reached about 7 % at that time. The next series of observations was conducted in the clonal seed orchard in Leżajsk in April 2001, analyzing the situation in the period from April 2000 to April 2001 (Fig. 7).

At that time the phenomenon of dying back , similar to that mentioned above, in the individual plots was again observed and the differences of per cent indexes between two "extreme plots" reached up to 8%.

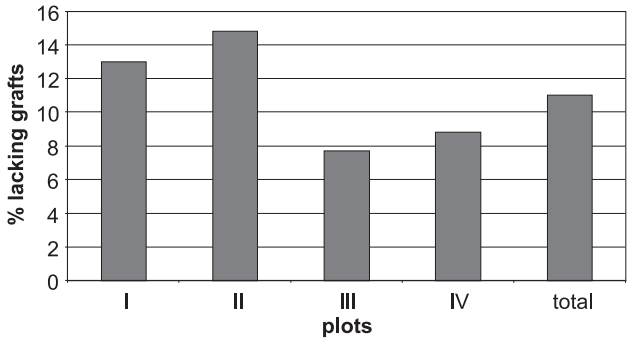

Fig. 6. Variation of the die-back of grafts in the four-year-old clonal seed orchard in the Forest District of Leżajsk in the period: April 1998–July 1999

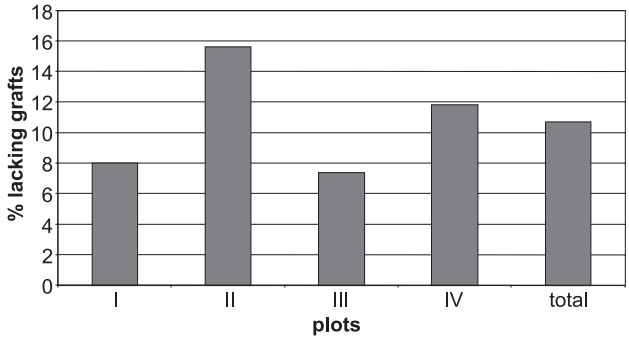

Fig. 7. Variation of the die-back of grafts in the six-year-old clonal seed orchard in the Forest District of Leżajsk in the period: April 2000–April 2001

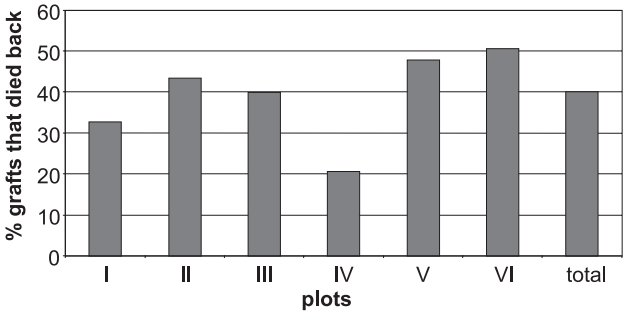

Fig. 8. Variation in number of the die-back of grafts in the eight-year-old clonal seed orchard of Douglas-fir in the Forest District of Łopuchówko according to the situation in July 2000

The situation in the clonal seed orchard in the Forest District of Łopuchówko was also examined. The inventory of losses was highest here in July 2000 (Fig. 8). The indexes of the grafts failure in the individual plots show a considerable variation between plots, coming up even to 30%.

Very crucial for drawing conclusions about the reasons of the die-back of grafts in the clonal seed orchards included into the observations is the fact that there exists a considerable interclonal variation. It appears in all units examined and is very clear. Detailed data concerning the die-back of grafts in 1998–2001, taking into account additional planting and overplanting, have led to the detailed analysis of the losses of every clone in the seed orchard of the Forest District of Leżajsk (Fig. 9a and 9b).

Investigations on vegetative propagation of Douglas-fir by rooting of cuttings

Rooting of cuttings could be another possibility for vegetative propagation of Douglas-fir.

Plants on their own roots, reproduced in the autovegetative way, are durable. However, rooting of mature ortets (plus trees) is difficult and unreliable. The age factor plays here an important role: the older a mother tree, the worse is the result of rooting. In a case of Douglas-fir, poor self- rooting of scions appears clearly when mother trees are more than 10 years old (Brix et al. 1973). Individual, genetically conditioned tree features influence the result of rooting.

However, rooting is not a perfect method of propagation. Plants obtained in that way may not be suitable for wood production due to their unfavorable cross-section that can be often observed.

However, irregularities of the shape are not an obstacle for abundant flowering and seed production. Hence thanks to rooting it is possible to obtain clone planting stock for creating and completing already existing clonal seed orchards and for establishing new ones.

The latest research on rooted progeny of individual Douglas-fir trees indicates that after the transfer from

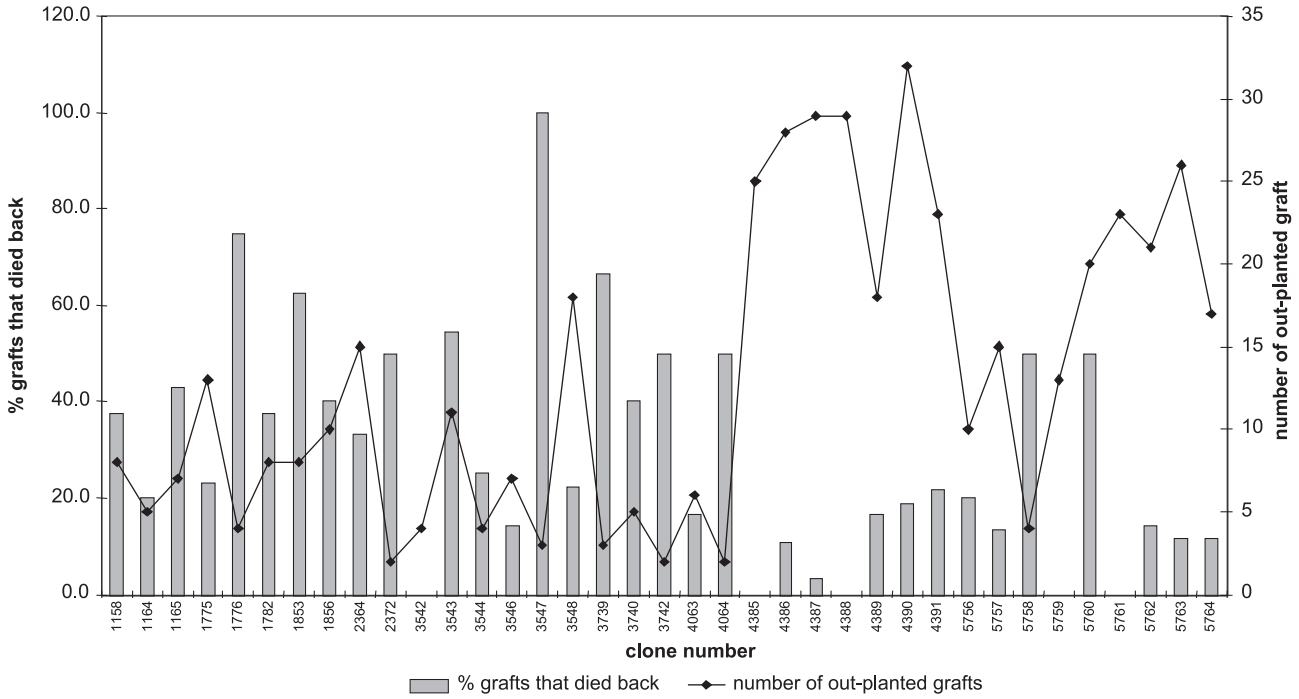

Fig. 9a. Variation in the die-back of grafts among clones in the six-year-old clonal seed orchard of Douglas-fir in the Forest District of Leżajsk in the period: 1.05.1998–1.05.2001

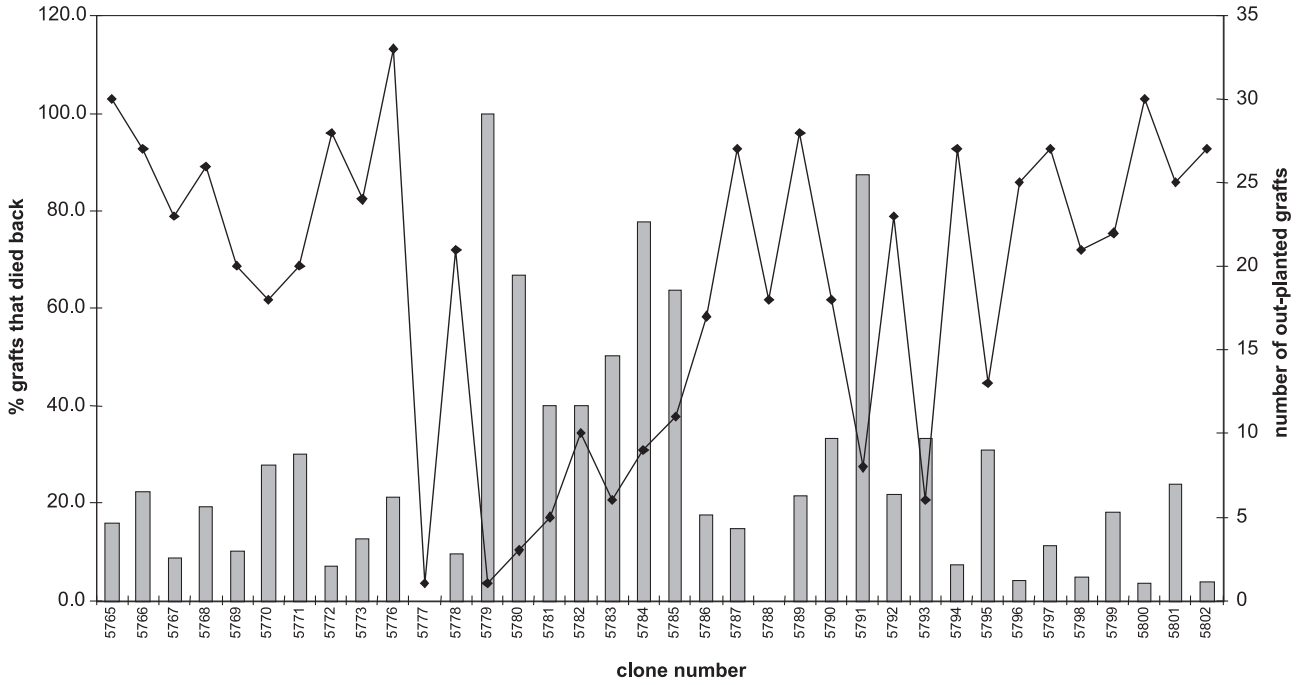

Fig. 9b. Variation in the die-back of grafts among clones in the six-year-old clonal seed orchard of Douglas-fir in the Forest District of Leżajsk in the period: 1.05.1998–1.05.2001

a shaded greenhouse to the open air all of the plants shift to ortotrophic growth phase (Ritchie et al. 1997).

So far, experience has not included in its range attempts at vegetative reproduction of already obtained Douglas-fir grafts themselves. In the case of other species, e.g. Norway Spruce, vegetative reproduction of mother plants that have been achieved through such reproduction influences the increase of percentage of the rooted shoots.

Attempts at rooting of Douglas-fir in Poland has been led by Professor Leon Mejnartowicz (Mejnartowicz 1990). He has conducted two series of experiments in rooting of scions taken from twenty-

-year-old mother trees. In his experiments he has used the following variant:
– two periods of shoot collection: February and March
– two lengths of scions: 7 cm and 20 cm
– two media: peat-sand and sand – perlite
– two variants of rooting-medium temperature: +15°C and + 25°C
– three hormones: IAA, IBA, NAA – in combinations: IAA, IAA+IBA, IAA+NAA, IBA, IBA+NAA, NAA
– two of scion planting: immediately after collection and 24 hours terms later
– two variants of seedlings: with incision of shoots before hormone application and without incision, only with the removal of cambium from about 5 millimeters of the tip of a shoot.

However, such a considerable number of variants and variables has not led to a positive result of the experiment. Hence production of callus in 1% of cuttings was the only result achieved. However, none of the cuttings produced roots. The author of this experiment points out three main sources of the failure:
– the advanced age of mother trees (20 years old)
– too large and too thick cuttings from the upper part of the tree crown
– too early termination of the experiment (after two months).

Own investigations conducted by the author show that obtaining cuttings from grafts, i.e. plants obtained through vegetative reproduction, has itself a great effect on the increase of percentage of rooting.

Shoots have been taken from grafts belonging to nineteen selected clones in the clonal seed orchard in the Forest District of Gniewkowo. As a control, cuttings collected from Douglas-fir seedlings in a four-year-old plantation have been used.

The experiment was set up in spring 1999 in five replicates, in a greenhouse with controlled watermist irrigation and artificial supplementary illumination.

Six thousand cuttings were taken for the experiment. As a variable, different variants of rhizoorigin stimulation have been applied. Three ingredients of the rotting-powder have been used: IAA-indoleacetic acid, kaptan, technical talc. Combination of those substances in various proportions allowed for the creation of six different variants:
1. IAA- 0,5 % + technical talc
2. IAA–1,0% +technical talc
3. IAA–0,5% + kaptan – 10% + technical talc
4. IAA–1,0% + kaptan – 10% + technical talc
5. kaptan–10% + technical talc
6. technical talc

The collection of cuttings in the plantation was done in March 1999. That material has been stored in controlled conditions with temperature of 3–4°C and the relative air at a humidity of 90–95%. Out-planting cuttings into the compost rooting-medium was done at the turn of April and May (Fig. 10).

After about two months, the cutting did not form roots (Fig. 11). However, we have found that Douglas-fir cuttings have an incredible ability to produce vegetation without producing a root system. As it has appeared, they are able to survive up to two years in the green state without taking nutrients from the medium.

In October 1999 formation of callus in 30% of cuttings has been observed. When these observations were continued up to 2001 it has been found that root initiation is not directly dependent on the extent to which callus is formed. That fact is confirmed also by foreign investigations, demonstrating simultaneously the importance of the hormone balance that is essential for cell differentiation inside the callus that is formed (Bhella 1973).

Relying on the proved causes of failures in attempts at Douglas-fir rooting conducted so far, it has been decided to keep the experiment as long as the cuttings placed in the compost medium are sufficiently viable. At present (June 2001) twenty six months have passed since the cuttings were planted out. All shoots dying back are systematically re-

Fig. 10. Cuttings taken from grafts in the clonal seed orchard

Fig. 11. Non-rooted cuttings after two months from outplanting in the rooting medium

Fig. 12. Rooted cuttings planted out into containers

moved. As a fungicide throughout the duration of the experiment, captan in the form of sprays every second week in spring and winter is applied. The index of rooted cuttings after two years from their planting out in the compost substratum has reached 1,3% – 77 individuals for the whole experiment (Fig. 12).

The best results have been achieved through the use of 1% IAA. However, the interclonal variation of the rooting ability became clearly visible.

It is important to point out that the age of cuttings should be linked with the age of the trees themselves from which scions for grafting have been taken. The age of mother plants from which the plant material has been taken for the rooting experiments is 100 and more years.

Taking into account the effect of the age factor on rooting, the obtained result of 1,3% of rooted cuttings should be regarded as a satisfactory one. Vegetative propagation of Douglas-fir plus tree by rooting of cuttings for the needs of clonal seed orchards of that species seems to be possible. For increasing the percentage of rooting, the best stimulating substance has to be chosen together with a careful selection of clones with easily rooting cuttings.

In April 2000 the next experiment was started in the clonal seed orchard in the Forest District of Gniewkowo consisting in the vegetative propagation of 42 Douglas-fir grafts (belonging to 20 clones) through aerial and soil layers.

Observations from spring 2001 indicate rooting of a few percent among of aerial layers (Fig. 13).

Fig. 13. Air-layer on a Douglas-fir graft

Conclusions

1. The crucial problem lies in the die-back of grafts in Douglas-fir clonal seed orchards in Poland, in other European countries and in North America. In the existing clonal seed orchards more that 50 % of grafts are eliminated in this way in the first 10 years of growth.
2. Research on the graft die-back conducted so far has not found the causes of that negative phenomenon. Excluded are fungal pathogens as a cause and they have alleged that abiotic factors like light frosts weakening grafts to some extent can have considerable influence on the occurrence of that phenomenon. Existence of genetic conditions influencing incompatibility of grafts with the rootstock is often presumed. Experiments conducted by the author so far point to the crucial importance of that factor among the causes of the observed losses.
3. One of the solutions to the existing problem could be a selection for grafting only of those clones whose grafts show the least incompatibility with the stock plants. This is possible judging by considerable variation in the dying-back of grafts of the individual clones.
4. There exists also the possibility of taking advantage of autovegetative methods of Douglas-fir propagation to supplement the losses in clonal seed orchards. Experiences in the propagation through rooting cuttings demonstrate a low percentage of rooting when they were taken from aged plus trees. Some hope arises because of satisfactory indexes of rooting of cuttings taken from grafts growing in clonal seed orchard and by Douglas-fir propagation through aerial layers on such grafts.

References

Bhella H. 1973. Root initiation and elongation in Douglas-fir *(Pseudotsuga menziensii* Mirb. Franco), stem cuttings as related to bud and cambial activity, Dissertation Abstracts International B, 34: 5, 1815; Order No. 73–25,342. ORS

Brix H., Barker H. 1973. Rooting studies of Douglas-fir cuttings. Information Report Pacific Forest Research Centre, Canada. No BC-X-87, 45.

Copes D. 1989. Bark scoring problem grafts in five Douglas-fir seed orchards: a care history, Research-Note-Pacific-Northwest-Research-Station--USDA-Forest-Service, No. PNW-RN-487, 12.

Mejnartowicz L. 1996. Rozmnażanie wegetatywne daglezji zielonej [Vegetative propagation of Douglas-Fir], Sylwan: 2, 25–37.

Mejnartowicz L. 1990. Stan prac nad rozwojem banku genów *Pseudotsuga menziensii* [Mirb.] Franco. Rozmnażanie wegetatywne daglezji zielonej [The state of researches on development of the gene bank of *Pseudotsuga menziensii* [Mirb.] Franco. Vegetative propagation of Douglas-fir]. Raport ID PAN Kórnik, str. 1–37.

Giannini R., Lambardi M., Vendramin G., Loreti F. 1988. Some obserwations on graft incompatibility in Douglas-fir *(Pseudotsuga menziensii* Mirb. Franco), International symposium on vegetative propagation of woody species. Acta Horticulturae: 227, 84–86.

Ritchie G., Keeley J., Ward P. 1997. Effects of shade and root confinement on the expression of plagiotropic growth in juvenile-origin Douglas-fir rooted cuttings, Canadian Journal of Forest Research, 27: 7, 1142–1145.