The Influence of Defined Rootstocks on Grafts of Norway Spruce (Picea abies L. Karst)*)

By G. H. Melchior**)

Federal Research Centre of Forestry and Forest Products,
Institute of Forest Genetics and Forest Tree Breeding

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Summary

The choice of defined rootstocks to increase seed yield and to shorten the juvenile period only had seldom been used in the past for forest tree breeding purposes and seed orchard practice.

In order to control the influence of rootstock on grafts, concerning survival, height growth, flushing and fruit production, scions of different trees of various ages from different provenances were grafted onto normal-growing and dwarfing monoclonal rootstocks. The following results were obtained concerning rootstock influence. There exist rootstocks with a high and low grafting compatibility for certain trees and populations. Rootstocks affect survival considerably and show a large variation in this respect.

The influence of rootstocks on the flushing of grafts could not be proved. There seems to exist an influence on height growth. Some clonal rootstocks seem to accelerate it, while others have an opposite effect. First effects on flowering behaviour have been observed on the grafts in a greenhouse and in a field trial in springtime 1982. Based on these preliminary observations it can be concluded that some rootstocks seem suitable for influencing grafts for the desired purposes. Further investigations and observations are necessary.

Key words: Norway spruce, dwarf rootstocks, grafting, survival, scions'growth, flushing, flowering.

Zusammenfassung

Die Wahl definierter Unterlagen, um die Saatgutausbeute zu erhöhen und die Jugendperiode abzukürzen, wurde bislang als Maßnahme in der Forstpflanzenzüchtung und Samenplantagenpraxis selten benutzt.

Mit dem Ziel, den Einfluß von Unterlagen auf Pfropflinge im Hinblick auf Überlebensfähigkeit, Höhenwachstum, Austreiben und Zapfenproduktion zu prüfen, wurden Reiser von Bäumen unterschiedlichen Alters verschiedener Provenienzen auf einklonige normalwachsende und Zwerg-Unterlagen gepfropft. Folgende Ergebnisse über den Einfluß der Unterlage wurden erhalten: Es existierten Unterlagen mit einer hohen und niedrigen Pfropfverträglichkeit für bestimmte Bäume und Populationen. Unterlagen beeinflussen die Überlebensfähigkeit beträchtlich und zeigen in dieser Hinsicht eine breite Variation.

Ein Einfluß der Unterlagen auf das Austreiben konnte nicht nachgewiesen werden, doch scheint ein Einfluß auf das Höhenwachstum zu bestehen. Einige Unterlagen scheinen es zu beschleunigen, andere haben einen gegenteiligen Effekt. Erste Wirkungen auf das Blühen wurden in einem Gewächshaus- und Freilandversuch im Frühjahr 1982 festgestellt. Aufgrund dieser vorläufigen Beobachtungen kann geschlossen werden, daß einige Unterlagen brauchbar erscheinen, die Pfropflinge in gewünschter Weise zu beeinflussen. Weitere Untersuchungen und Beobachtungen sind notwendig.

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**) Address: Sieker Landstr. 2, 2070 Grosshansdorf 2, Federal Republic of Germany

1. Introduction

Norway spruce is one of the most important forest tree species in Germany, because of its relatively quick growth, the area covered and the possible financial gain. Therefore, a sustained forestry with this species is a necessity, which depends amongst other things on a continuous seed supply of defined well growing provenances and improved cultivars. The use of seed orchard seeds represents a possibility to supply improved reproductive material. Unfortunately seed orchards of Norway spruce flower at more or less large intervals and often bear fruits in small quantities. Therefore, techniques which increase the fruiting frequency and cone production are needed for a continuous seed supply and seed orchard practice.

Many techniques are known to stimulate flower and fruit production — also in forest trees (Sweet 1975, Krusche *et al.* 1977). But in general the increase of the ability to fruit diminishes after a certain time and the treatment has to be repeated. Fruit breeders have solved this problem by rootstock breeding. So for most fruit trees the desired rootstocks to provoke a certain effect in the grafts are available.

In forest trees grafting is mostly done onto young seedlings of known or unknown parentage. In consequence not only the influence of the rootstock upon the scion is unknown but also a desired influence of a certain rootstock cannot be repeated. First results about the influence of defined rootstocks in forestry could be obtained in Norway spruce (Bryndum 1965). Langner's experiment established in the fifties on the use of dwarf Norway spruce (ornamental genotypes) showed a broad variation concerning dependence of various interesting characters on the rootstock (Krusche *et al.* 1976, 1977). To find a broader base on the variation of influence of defined and reproducible rootstocks upon scions in Norway spruce appropriate experiments have been started nine years ago by the production of clonal rootstocks. Some results of a six year old grafting experiment onto such rootstocks are reported here.

2. Materials and Methods

2.1 *The scions* were collected one to seven weeks before grafting and stored in closed plastic bags at a temperature of about -5^0 C. The treatment within each experiment was

Table 1a. — Specification and number of provenances, age classes, trees, ramets, and rootstocks grafted within each treatment. C = CSSR; R = Romania; D = Denklingen; S = Schwarzwald; W= Westerhof; Su = Sundmo; T = Trittau.

Provenance	C	R	D	S	W	Su	T	T	T	T	T	T
Age	8	8	20	20	20	20	71	96	36	40	71	96
Trees	5	5	3	3	3	3	10	10	5	5	5	5
Ramets	4	4	6	6	6	6	20	20	5-8	5-8	5-8	5-8
Rootstocks	9	9	17	17	17	17	7	7	16	16	16	16

Table 1b. Tested rootstocks.

No	Type	Name
1	Dwarf (D)	Picea glauca 'Conica'
2	D	P. abies 'Nidiformis'
3	D	P. abies 'Pygmaea'
4	D	P. abies 'Humilis'
5	D	P. abies 'Clanbrassiliana'
6	D	P. abies 'Conica'
7	D	P. abies 'Ohlendorffii'
8	D	P. abies 'Pyramidalis Robusta'
9	D	P. abies 'Merkii'
17	D	P. abies 'Pumila Glauca'
18	D	P. abies 'Pygmaea'
19	D	P. abies 'Nana Compacta'
10	Normal (N)	P. abies, Th 1332
12	N	P. abies, Th 1320
13	N	P. abies, Th 1323
15	N	P. abies, We 49/9/4
16	N	P. abies 'Columnaris'

identical. More details of the scions are summarized in *Table 1a.*

2.2 *The rootstocks* were clonal material rooted in the institute's nursery. They originated from commercial dwarf material, dwarf material from the institut's "Arboretum Tannenhoeft", and normal growing trees of ages between 20 to 70 years. In the grafting year the rootstocks were 3 years old. The specification of the rootstocks is presented in *Table 1b.*

2.3 *The grafting methods* were Side Veneer Grafting and Bark Grafting with Triangular Scions. The needles from scions and rootstocks were removed on a length of about 5 cm. Grafting on normally growing rootstocks was done on the main shoot and in the dwarfs on the thickest one in February and March 1977 after the rootstocks started flushing. In general, the scions had diameters of about 0.3—0.6 cm. Rootstocks with a thickest shoot of 0.5 cm or more were used. Two grafters with much experience in the applied techniques realized the grafting work.

2.4 *After grafting care* in the greenhouse and nursery consisted of regular water supply and fertilization by flooding and removing of the shoots of the rootstocks 3 to 6 weeks after grafting at intervals of one to two weeks after the scions had flushed. The grafts were put in the nursery at about the end of May after the frost risk was over.

2.5 *Design:* To avoid large handling expenses and risk of errors by the large number of grafts (*Table 1a:* product of trees, ramets and rootstocks) most parts of the experiment were put in rows with the graft combinations vertically to the main gradient in the greenhouse or nursery. One experiment had been carried out as a completely randomized block design with two replications.

2.6 *Data taking* and *evaluated traits:* The survival was calculated in springtime and autumn. The height of the scion was measured in the autumn beginning with the year of grafting. Flushing was assessed by 5 scores at intervals of one week (1: beginning of flushing by the opening of the bud scales, 2—4: various consecutive stages of bud break and extension growth, 5: clear extension growth of the new shoot, see Volkert *et al.* 1966). The number of ramets with male strobili was counted.

2.7 *The evaluation* of the traits was made by analysis of variance and contingency tables.

3. Results and Discussion

3.1 *Survival*

The survival of a graft depends on various factors: The influence of the provenance of the ramet plays a role. Some 5 to 7% are due to this source of variation in young provenances. Differences exist between trees of the same provenance *(Table 2).* The variance components between 0 and 45% indicate high dependence on this source of variation *(Table 3).* The variation seems to increase with increasing age of the ortets. About 20—50% of the variation are due to the rootstocks *(Table 3).* Provenances, trees and especially rootstocks are the main sources of variation affecting the survival of grafts, in many cases significantly *(Table 3).* Also the grafters' influence on the survival could be proved *(Table 2).* This suggests the necessity for proper choice and training of grafters to improve the outcome.

Table 3. — Results on the influence of trees and rootstocks on the survival of grafts. Level of significance: * = 5%; ** = 1%; n.s. = non significant.

Age of trees	Trees DF	Trees VC%		Rootstocks DF	Rootstocks VC%		Error DF	Error VC%
8	4	3.3	n.s.	8	25.6	+	32	71.1
8	9	13.6	++	8	29.3	++	72	57.1
20	2	0		16	22.2	n.s.	32	77.8
20	2	1.7	n.s.	16	8.3	n.s.	32	90.0
20	2	11.1	+	16	34.7	++	32	54.2
20	2	26.0	++	16	41.7	++	32	32.3
36	4	33.6	++	16	42.2	++	64	24.2
40	4	3.7	n.s.	16	47.3	++	64	49.0
71	4	11.5	++	16	20.8	++	64	67.7
71	9	40.0	++	6	26.0	++	54	34.0
96	4	19.9	++	16	31.1	++	64	45.0
96	9	45.1	++	6	21.7	++	54	33.2

The age of the ortets from where the scions originated seems to play an important role on the survival of the grafts. Unfortunately we could not use the same provenances at different ages and the effect of the provenance and age classes could not be differentiated *(Table 1a:* 'T' is not indigenous but a mixture of various provenances which were planted in different years). *Figures 1—3* clearly show that incompatibility between rootstock and scion finds its strongest expression in the second vegetation period in grafts from younger ortets (till 20 years old) and

Table 2. — Survival (%) of grafts originating from ortets of an age of 71 years, made by 2 grafters (x, y).

Root-stock No.	1 x	1 y	2 x	2 y	3 x	3 y	4 x	4 y	5 x	5 y	6 x	6 y	7 x	7 y	8 x	8 y	9 x	9 y	10 x	10 y	$\bar{x}$ x	$\bar{x}$ y
1	80	70	40	50	30	60	0	30	70	60	0	10	0	10	50	40	20	30	40	50	33	41
2	20	60	20	20	10	20	10	10	0	10	0	0	0	10	0	20	10	10	0	40	7	20
3	40	50	0	10	0	0	0	10	0	20	0	0	0	0	0	0	10	20	10	20	6	13
4	20	40	0	10	0	10	0	20	0	10	0	10	0	0	0	10	0	10	0	10	2	13
5	50	70	30	70	30	70	10	40	20	50	0	0	20	30	30	50	0	20	0	20	19	42
6	10	20	10	0	10	0	0	20	0	10	0	0	20	0	0	30	0	10	20	50	7	14
7	30	70	0	30	0	60	0	0	30	20	0	0	10	10	10	10	10	20	10	50	10	27
$\bar{x}$	36	54	14	27	11	31	3	19	17	26	0	3	7	9	13	23	7	17	11	34	12	24

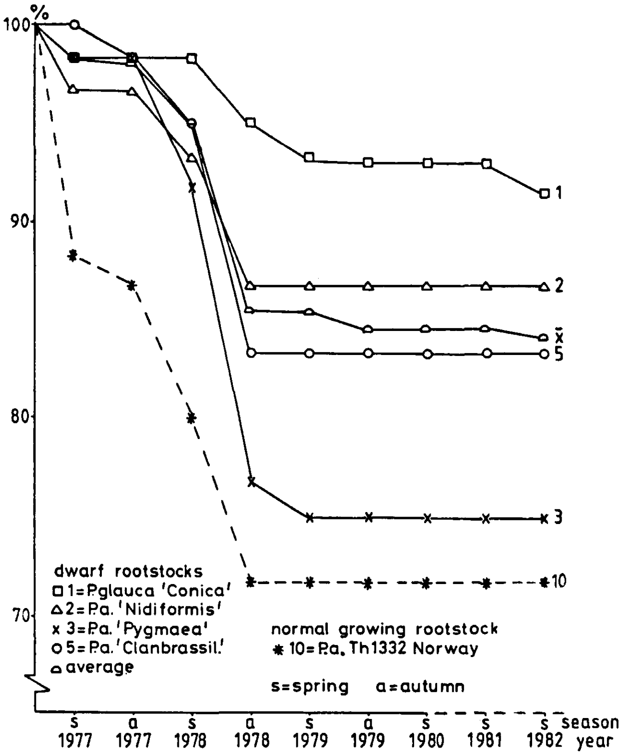

Fig. 1. — Survival of scions of 8 year old Norway spruce on different dwarf and normal growing rootstocks.

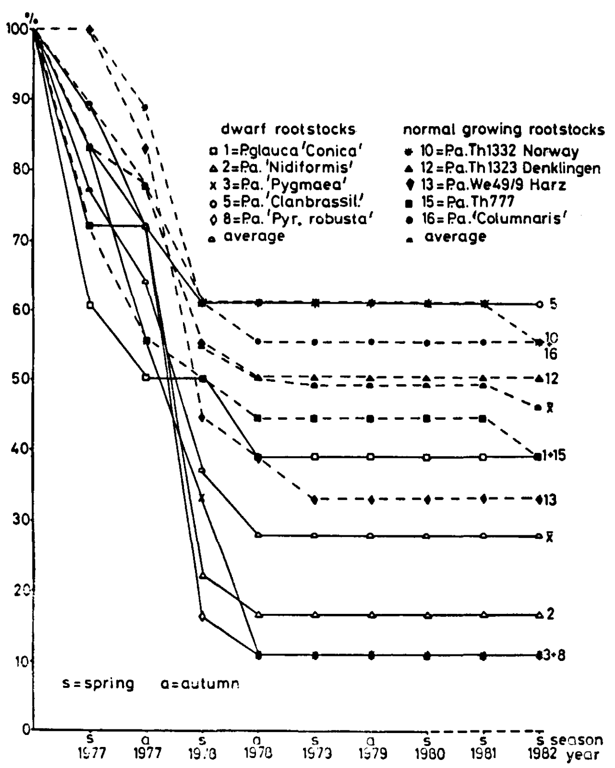

Fig. 2. — Survival of scions of 20 year old Norway spruce provenances on different dwarf and normal growing rootstocks.

earlier when the scions originated from older trees. A limiting value for mortality will be reached at the end of the second vegetation period after grafting. On the other hand a decrease of survival in Norway spruce grafts seems to be after 8 and before 20 years of age of the ortets *(Figures 1, 2).*

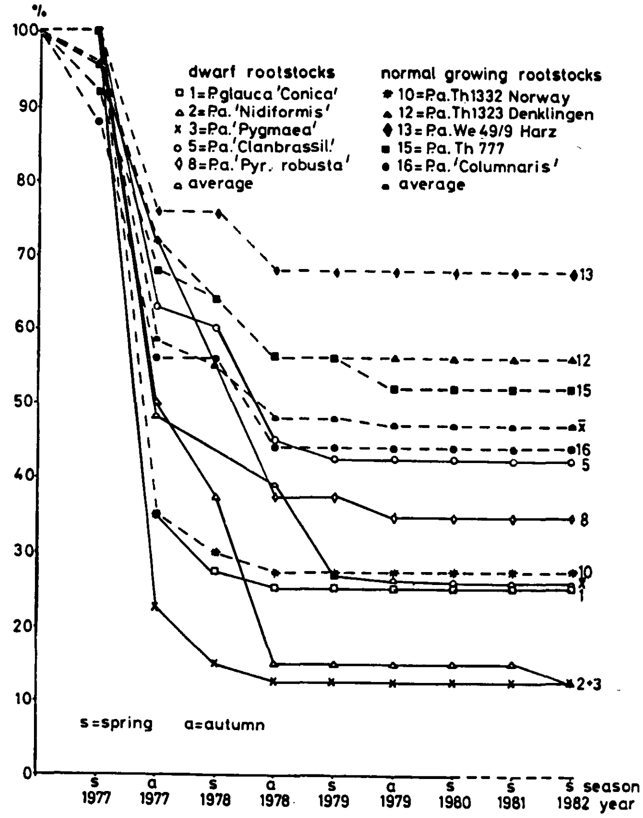

Fig. 3. — Survival of scions of 71year old Norway spruce on different dwarf and normal growing rootstocks.

Many techniques found as optimum for grafting of *Picea abies* e.g. top grafting, (Tzschacksch 1969) could not be applied to the very different types of dwarf and normal growing rootstocks. Therefore, optimizing such methods especially for grafting onto dwarf rootstocks might increase the survival considerably.

3.2 *Length growth of the scions*

The length of the scions as dependent on the trees and rootstocks is shown in *Figure 4* using the Black Forest 'Schwarzwald' provenance as an example. First the figure shows the higher survival of scions grafted onto normal growing rootstocks (No. 10—16) where not a single tree had to be presented by only one ramet as in the group of dwarf rootstocks (e.g. No. 8). That is the reason also for difficulties in the evaluation. The standard deviation shows a small to broad variation within and between trees (e.g. rootstocks 5, 10: trees 1 and 2). That is true also for rootstocks and groups of rootstocks and for other provenances and age classes. In general, scions of younger provenances showed a greater length than scions from older ones, and on dwarf rootstocks a greater length was reached than on normal growing ones *(Table 4).*

3.3 *Flushing behaviour*

Figure 5 presents the flushing behaviour of 7 provenances of equal and different age classes, which shows large differences in development between them. The slowest beginner is from Romania, the quickest originates from the Black Forest which conserves its advantage till flushing stage 5. Other provenances, as for example 'Denklingen' (D) also a slow beginner, runs through the next stages quickly and after a month nearly overtakes the quick flushing Black Forest provenance. On the other hand, the

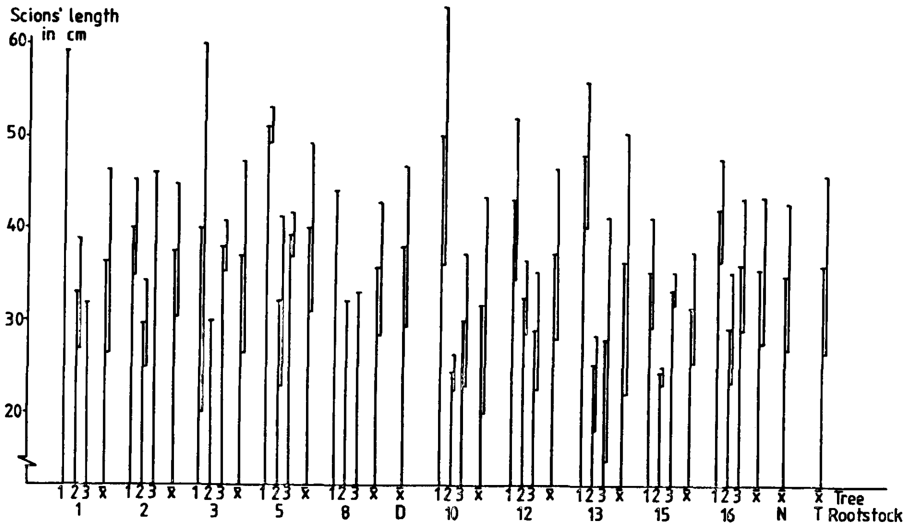

Fig. 4. — Dependence of length of scions on trees and on dwarf (D) and normal growing (N) rootstocks in provenance „Schwarzwald" three years after grafting. The standard deviation is mentioned.

provenances from southeastern Europe conserve a slow flushing behaviour till stage 5. An influence of the rootstock on the flushing could not be observed yet. Thus, flushing in this early stage depends primarily on the grafts, on the provenance and tree from which the scions originate.

3.4 *Flowering*

First male flowering of the grafts both in greenhouse and in the field trial has been observed in spring time

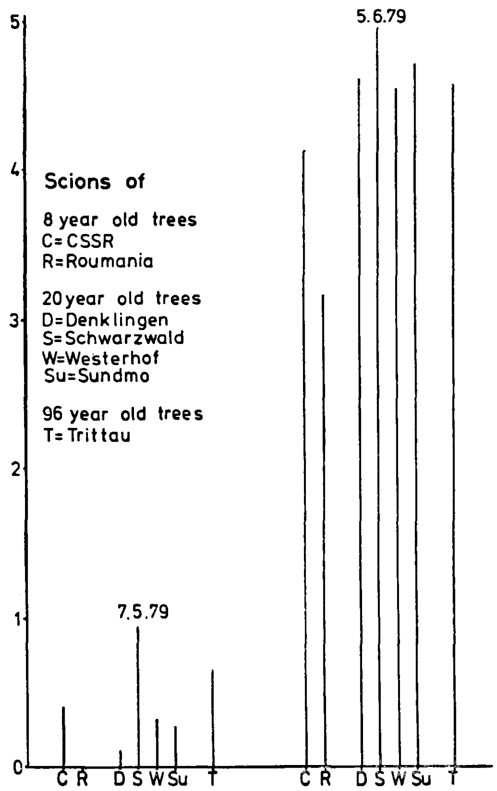

Fig. 5. — Flushing of grafts of trees of various provenances of different ages on 8 dwarf and 1 normal growing rootstocks. The weighted means on the assessed phases of flushing (0—5) on two dates (times) are presented.

Table 4. — Length of the scions two years after grafting in relation to provenances/age classes and 11 selected rootstocks.

Root-stocks	Age classes/provenances 8	20	36	40	71	96
1 (D)	34.0	39.7	34.7	32.1	35.7	32.4
2	36.0	38.8	38.0	35.3	32.2	32.0
3	35.8	35.0	32.4	33.5	37.2	31.4
5	33.8	39.1	33.6	34.3	33.2	31.6
8	-	36.3	28.8	28.2	31.2	27.4
19	-	32.8	31.1	29.3	30.1	26.2
10 (N)	33.1	34.9	33.9	32.8	30.2	27.7
12	-	35.1	30.5	32.7	32.4	33.2
13	-	38.3	29.2	33.3	32.9	26.5
15	-	31.9	27.4	30.9	27.4	24.9
16	-	33.6	26.9	31.0	28.9	27.3

1982, five years after grafting. Seven of the 68 graft combinations on dwarf rootstocks flowered with one to eight ramets (total 16) and three of the 26 combinations on normal growing ones with one ramet each (*Table 5*). Two combinations on dwarf rootstocks (No. 1, 5) flowered in more than one age class with a total of five and eight ramets. The combinations with scions from older ortets showed more flowering ramets than younger ones. The scions of the 19 flowering grafts originated from nine trees out of a total of 40 ortets.

In the field trials on two sites with different climatic conditions concerning temperature during the vegetation period flowering started only on the warm site (18° C : 14° C in the vegetation period) with 3 ramets. The same trend could be observed in one greenhouse/nursery trial in Schmalenbeck. Only the group with higher temperature greenhouse treatment in May/June showed flowering

Table 5. — Time of flower initiation in Norway spruce grafts following exposure to different temperature regimes in the greenhouse and nursery during spring 1981. The number of flowering ramets has been mentioned. Temperature intervalls for March/April: 20—30, April/May: 20—30, May/June: 20—37° C. Control (March—June): −4—+30° C.

Rootstocks	Greenhouse March/April	April/May	May/June	Nursery Control	Total
Dwarf: 12	0/193 0%	0/194 0%	14/175 8%	0/249 0%	14/811 1,7%
Normal: 5	0/59 0%	0/59 0%	2/57 3,5%	0/79 0%	2/254 0,8%
Total: 17	0/252 0%	0/253 0%	16/232 6,9%	0/328 0%	16/1065 1,5%

Table 6. — Ranks of survival, height and flowering of grafts in relation to age classes/provenances and 11 selected rootstocks out of 17 rootstocks used as mentioned in *Table 1a.* A high survival percent, a low height and a high number of flowering ramets correspond to a low rank and the desired property of a rootstock.

Rootstocks		Survival 1982						Height 1979						Flowering 1982					
Type	No	8	20	36	40	71	96	8	20	36	40	71	96	8	20	36	40	71	96
Dwarf	1	1	3	2	2	12.5	3	5	16	15	9	15	15	5	9	1	9	3	2
	2	2	12,5	17	17	16.5	15	8	14	17	17	9	14	5	9	10	9	4	10.5
	3	6	15	13	13	16.5	11.5	7	7	9	15	17	11	5	9	10	9	12	10.5
	5	4	1	4.5	4	5	7	4	15	13	16	13	12	5	9	10	9	2	3
	8	-	17	9	13	7	10	-	9.5	3	1	7	8	-	9	10	9	12	10.5
	19	-	12.5	3	9	6	13	-	2	6	2	4	3	-	9	10	9	12	10.5
Normal	10	7	4	8	6.5	10.5	2	2	6	14	12	5	9	5	9	10	9	12	1
	12	-	7	10.5	5	2	6	-	8	5	10.5	10	17	-	9	10	9	12	10.5
	13	-	5.5	1	1	1	1	-	13	4	13.5	12	4	-	9	2	9	12	10.5
	15	-	10.5	7	8	3	9	-	1	2	5	1	2	-	9	10	9	1	10.5
	16	-	2	10.5	3	4	4	-	3	1	6	2	7	-	9	10	9	12	10.5

ramets. March till mid-May treatments and nursery control did not flower. So, at the same time we could find an approximation for the time of flower initiation which took place in 1981 between the 15 of May and the 15 of June *(Table 5).*

4. Conclusions

For the seed orchard practice we need a rootstock which increases survival, reduces the height growth and stimulates flowering and cone production of a graft (see Heybroek *et al.* 1976) at a time when late frosts can no longer damage the flowering strobili. Unfortunately this latter intention to influence the flushing behaviour could not so far be observed on the tested clonal material. Although the ortets of the clonal rootstocks used vary considerably concerning this character, the inherited provenance and/or tree effects could not be influenced by one rootstock from the tested 17. We have to consider this problem as open. But if we rank the results of survival 1982, length of scion 1979 and flowering 1982 in this way that low values correspond to a high survival percent, short scions and a high number of flowering grafts *(Table 6)* some preliminary indications on the possible suitable clonal rootstocks can be obtained, which underline our results (Krusche *et al.* 1976, 1977). The high survival of grafts on dwarf rootstocks of *Picea glauca* 'Conica' and *Picea abies* 'Clanbrasiliana' an early start of flowering and a relatively high number of flowering ramets on these rootstocks indicate that both should be especially observed. The influence on flowering, fruit setting and compatibility will be the most important criteria for recommending the first standard Norway spruce rootstocks in the future. Other criteria of less weight are shoot growth and flushing.

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References

Bryndum, K.: The effect of the rootstock upon the height growth of Picea abies grafts. Det forstl. Forsøgsvesen i Danmark **29**, 163—171 (1965). — Heybroek, H. M. and T. Visser: Juvenility in fruit growing and forestry. Acta Horticulturae No. **56**, 71—80 (1976). — Krusche, D. and Melchior, G. H.: Unterlagenwahl als Mittel zur Beeinflussung der Blüte und des Samenertrages bei Fichte. Silvae Genetica **25**, 216—222 (1976). — Krusche, D. and Melchior, G. H.: The choice of rootstock as a mean to stimulate flowering and increase the cone yield in Norway spruce grafts. Third World Consultation on For. Tree Breeding, Canberra/Australia 1977, Vol. 2, 1079—1087, Canberra (1978). — Sweet, G. B.: Flowering and seed production. In: R. Faulkner: Seed orchards. For. Comm. Bull. No. 54, 72—82 (1975). — Tzschacksch, O.: Untersuchungen zur Verbesserung der Anwuchsergebnisse von Gewächshauspfropfungen der Baumart Fichte *(Picea abies* (L.) Karst.) Diss. Inst. f. Forstwissenschaften Eberswalde, Graupa (1969). — Volkert, E. und Schnelle, F.: Erläuterungen zur Beobachtung des phänologischen Termins 'Nadelentfaltung' bei der *Picea abies.* Arboreta Phaenologica Nr. 8, 1—9 (1966).

Protoplast Research in Woody Plants*)

By M. R. Ahuja

Federal Research Center for Forestry and Forest Products,
Institute of Forest Genetics and Forest Tree Breeding,
Sieker Landstraße 2, D-2070 Grosshansdorf,
Federal Republic of Germany

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Abstract

The status of woody plant protoplast research is reviewed. Protoplasts have been isolated and cultured from several woody plant genera of angiosperms and gymnosperms. Sustained cell divisions, colony/callus formation have been observed in the protoplast cultures of a few woody species. With the exception of *Citrus* species, plant regeneration from the protoplast cultures of other woody plants has not been achieved so far. Protoplast fusions have been attempted in a few woody genera. Somatic hybridization techniques seemingly offer new options for combining genotypes, isolation of novel and new genotypes, understanding of growth and differentiation, and genetic improvement of woody plants.

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