Observations on induced polyploidy in some conifers

(Pinus silvestris, P. contorta, Picea abies and Larix sibirica)

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In the forties rather comprehensive series with colchicine treatments were performed in different coniferous species at the tree breeding station, Ekebo. The purpose was to ascertain whether valuable tetraploid or triploid populations could be obtained in this way. Germinating seeds were treated with C-solutions of different concentrations and during different exposure times. Concentrations of 1.25%—1.5% during 2 to 4 days were most efficient, giving high proportions of seedlings with strong morphological deviations of wellknown types (KIELLANDER 1946, 1950).

After the treatment the material were cultivated in ordinary ways, the first period in the greenhouse, the second in the outdoor nursery, and were lastly transplanted to arboretum quarters.

In all stages the mortality has been high, the growth weak and the form of growth more or less abnormal. Per se the vegetative performance of the Co-generation is of little consequence. In principle the Co-individuals arc mixoploids, and their possible value is as producers of diploid gametes. Thus, the chromosome numbers in the subepidermic cell layer, from which the sporogeneous tissue is derived, become decisive. It means that the Co-individuals attain real interest first when flowering appears and the characteristics of the gametes can be studied and C,-families be possibly raised. Not until about 30 years after the treatment do these materials commence to enter the flowering stage. In spite of the fact that the number of flowering C,-trees is still low and the studies performed rather superficial, a short report might be motivated considering the length of time during which these experiments have been going on.

Materials

In 1974 the materials mentioned below are all that is still alive of the entire series of treatments.

Pinus silvestris: treatment 1945, 7 trees from the same source of seed (South-Swedish), all weak and abnormal (Fig. 1).

Pinus contorta: treatment 1945, 2 trees very small but rather well-shaped.

Picea Abies: treatment 1941, 30 trees, all of C_0-type,
treatment 1942, 17 trees, 16 of C_0-type, 1 normal,
treatment 1943, 2 trees, all of C_0-type,
treatment 1945, 30 trees, 15 of C,-type, 15 normal.

These trees represent a number of seed sources, both Nordic and Central-European. The trees of C,-type show strongly retarded growth. The tallest one has a height of 9 m (Fig. 2), but most of them lie between 3 and 4 m and some are as low as 0.5—1 m. The untreated controls have obtained heigths of 10—12 m.

Larix sibirica: treatment 1945, 7 trees, all of C,-type. Series with other larch species as well as F, species hybrids were also carried out, but they were lost at early stage. The cause to this was not only the toxicity of the colchicine but damping of contributed also.

Fig. 1. — ***Pinus silvestris*, C_0.** Treatment in 1945. Photo in 1975.

Now and then branches of normal type have appeared on the C,-individuals, particularly on spruce, but such branches have successively been removed, and today the trees of C,-type of all species give a rather homogenous impression. Moreover, the C_0-trees, particularly of spruce, vary considerably as regards needle characters and form of growth, but they all deviate strongly from the normal diploid type.

At the early nursery stage the 2n-numbers were ascertained in the root meristems. It was stated that uninfluenced seedlings had 2n = 2x, whereas influenced ones often 2n = 4x. Individuals with 2x- and 4x-cells as well were also found. Measurements of the length of the stomata cells were also performed, and demonstrated that influenced seedlings often had longer stomata cells — 32 units — than uninfluenced ones — 25 units —. Correspondence between the 2n-numbers in the root meristems and the length of the stomata cells was not always present (KIELLANDER 1946, 1950).

At the late nursery stage 2n-countings were performed also in the needle primordia. In seedlings of clear C_0-type, only 4x-cells were seen.

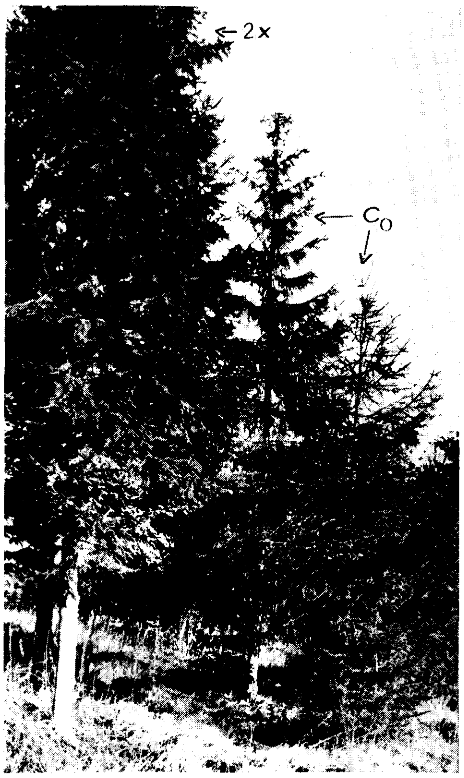


Fig. 2. — *Picea Abies*, C_0. Treatment in 1941. Photo in 1975.

In 1973, 2n determinations in the needle primordia were performed for all remaining C_0-trees of *Pinus silvestris* and *Larix sibirica,* and for three trees of *Picea Abies,* chosen at random. Only 4x cells were found. Thus, the induced tetraploidy had been maintained for a period of about 30 years. The suspicion, expressed by Straub (1941), that induced polyploidy in woody plants might be of temporary nature only and fairly soon disappear owing to "autorestitution" has been proved to be wrong for conifers as well as for broad-leaved species (Johnsson 1949, 1956).

However, it is far from excluded that further studies might well reveal that certain individuals are to a certain degree mixoploid.

The flowering

In the spring of 1971, male inflorescences appeared on one C_0-tree of *Pinus silvestris,* and the following spring on still another *silvestris-* and on one *contorta*-tree. In 1973, also one C_0-tree of *Larix sibirica* produced quite a number of male inflorescences and furthermore two female inflorescences. The year after, another C_0-larch carried male inflorescences, and the first one had, in addition to male, also 18 female inflorescences. Already in earlier years the last-mentioned tree had had some few male- and female inflorescences. In the same year (1974) some few inflorescences were seen on the two tallest C_0-trees of *Picea Abies.* These trees belong to a series of treatment performed in 1941 and are of South-Swedish origin. One of them carried 17 female inflorescences and some few male ones, the other having one female inflorescence.

The pollen character

Both the pine species and *Picea Abies* produce pollen of a very striking abnormal and similar appearance. This pollen is particularly characterized by a large portion of the grains having supernumerary air sacs, often three, sometimes four sacs of about equal size, not seldom two larger and two smaller ones. More rarely, there is a single "air buoy" all round the grain *(Figs 3 and 4).* Pollen of this general type has been also observed by Andersson (1947) in asyndetic *Picea Abies,* by Zinnai (1953) in C_0-*Pinus densiflora,* by Kim *et al.* (1967) in C_0-*P. rigida* and by Chira (1969) in *P. edulis* as a consequence of low temperature.

This C_0-pollen has also larger dimensions than the normal, haploid pollen, which is shown in *Table 1,* concerning the diameters between the air sacs for the pines.

The overlapping is considerable but the differences between the mean values of 2x and C_0 are quite clear. The percentages of abnormal grains (supernumerary air sacs) show a strong individual variation with much lower values for C_0-*contorta* than for C_0-*silvestris.* The abnormal grains in C_0-*contorta* generally have three well separated air sacs of equal size. Grains with two larger and two smaller sacs, which are most common in C_0-*silvestris* are almost completely lacking in C_0-*contorta.* The relations between dis-

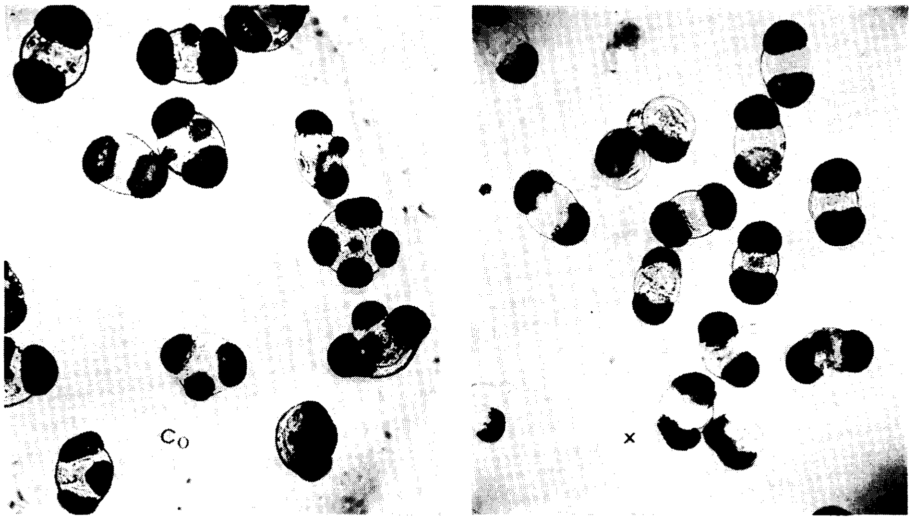


Fig. 3. — *Pinus silvestris,* pollengrains of C_0, left, and 2x-control, right, x 160.

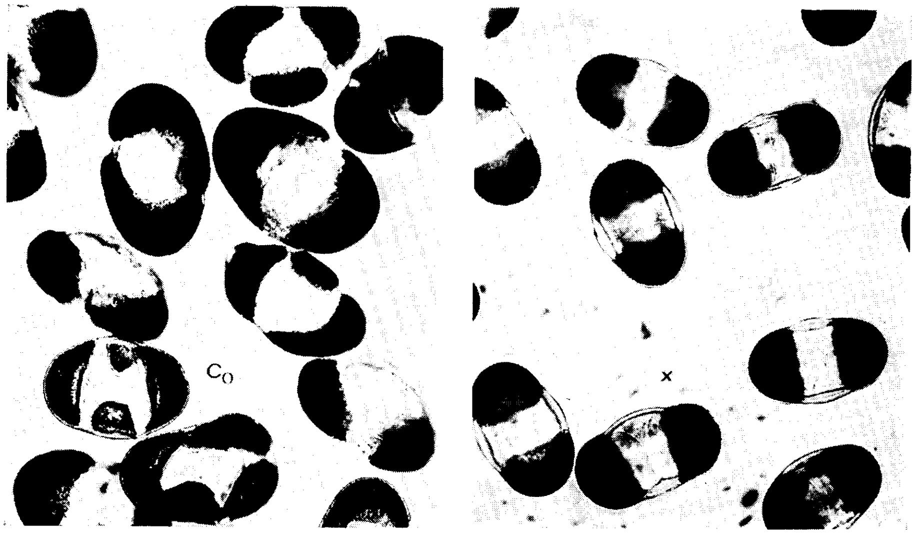


Fig. 4. — *Picea Abies*, pollengrains of C$_0$, left, and 2x-control, right. x 160.

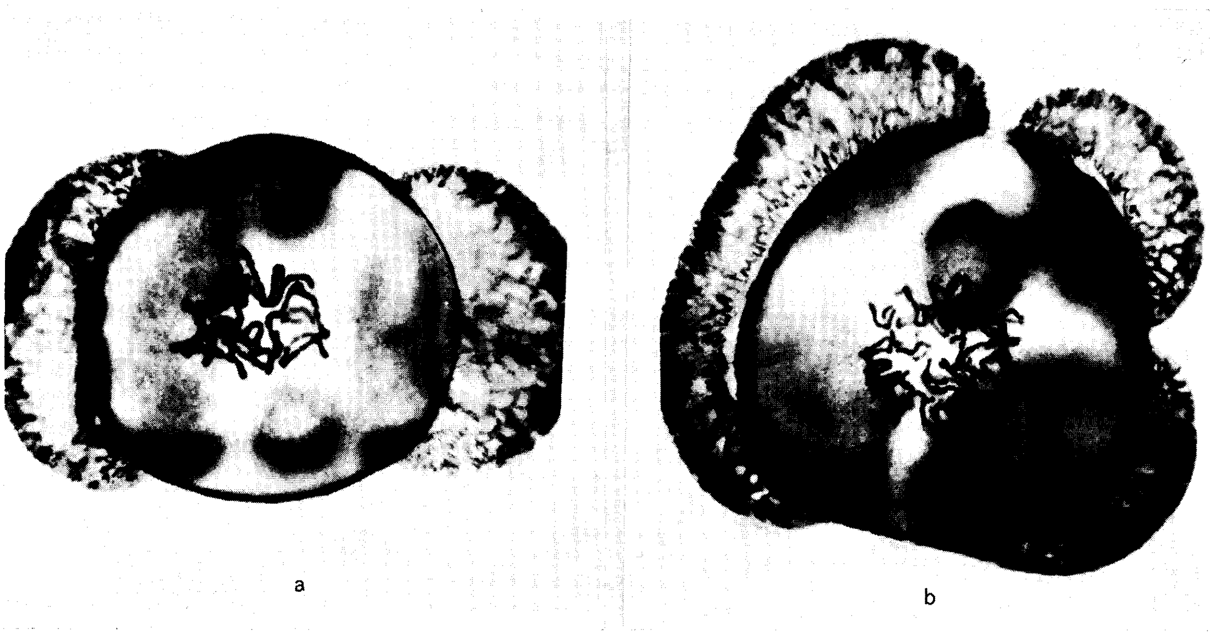
Table 1. — The pollen grain diameters between the air sacs for the the pines (1 unit = 12 mμ)

	Number of grains in different classes											$\bar{x}$	Rel. figs.	% abn. grains
	17	18	19	20	21	22	23	24	25	26	27			
P. silvestris														
2x, control	7	28	41	20	4							18,9	100	0
C$_0$—2	2	7	19	34	21	10	4	3				20,3	107	60,2
C$_0$—5		1	3	13	28	12	14	12	11	5	1	22,3	118	95,0
P. contorta, C$_0$			1	7	9	20	29	19	11	3		22,8	121	20,1

tributions and mean values for the length of the pollen grains, including air sacs, are very much the same as the relations of the grain diameters.

In spite of these striking pollen grain abnormalities the meiotic divisions seem to pass rather regularly, even if lagging chromosomes are of common occurrence. The tetrads are generally quite regular. In some cases it has been possible to state the number of chromosomes in the first pollenmitosis with satisfactory precision. Most of these have shown n = 2x (*Fig. 5a*) and in some few particularly large grains n = 4x has been counted (*Fig. 5b*). In vitro the C$_0$-pollen has germinated as well as the haploid pollen (*Fig. 6*).

On account of the irregularities of the pollen grains of C$_0$-*Picea Abies*, it has been impossible to measure the dia-



a

b

Fig. 5. — *Pinus silvestris*, pollenmitotis, a, showing n = 2x, b, n = 4x, x 1,400.

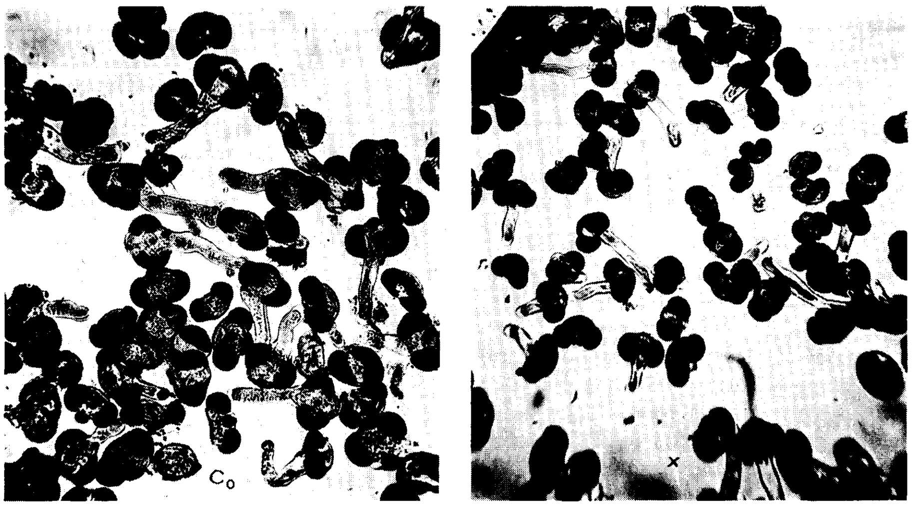


Fig. 6. — *Pinus silvestris*, germinating pollengrains of C_0, left, and 2x-control, right. 48 h., 24° C. x 80.

meters with satisfying accuracy, and consequently only their length including air sacs has been measured. On an average the C_0-grains are only 4% longer than x-grains, 50.6 units as compared with 48.5. However, the variation is considerably broader, the coefficient of variation being 0.09 as against 0.04, which is illustrated in *Fig.* 7.

The C_0-pollen grains of *Larix sibirica* are exceptionally large in comparison with the normal, haploid pollen grains. The mean diameter is not less than 37% larger, 39.5 as compared with 28.8 units *(Fig. 8)*. The overlapping is quite insignificant, the C_0grains varying between 30 and 48 units, the haploid grains between 25 and 32. The coefficient of variation is 0.08 for the C_0-pollen as compared with 0.06 for the haploid pollen.

The fertility of C_0-trees

In 1971 and 1972 diploid mother trees were pollinated with C_0-pollen in both pine species. In these crosses two diploid mothers and two C_0-trees of *P. silvestris,* and two diploid mothers and one C_0-tree of *P. contorta,* were involved. The outcome of these crosses is summarized in *Table 2.*

The proportions harvested to pollinated inflorescences, 29% and 35% respectively — is not so low per se, even if they are lower than the usual outcome for pine crosses, which is most often 50—75%. The number of seeds per cone

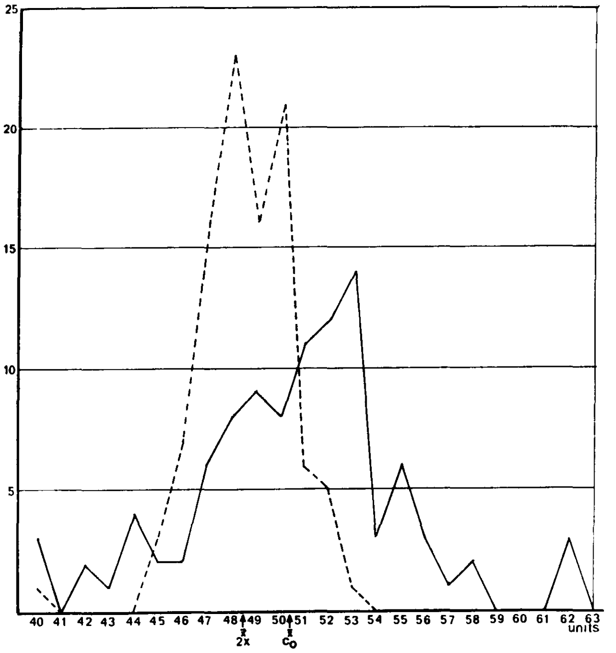


Fig. 7. — *Picea Abies,* frequency distribution of pollen grains of C_0, solid line, and 2x-control, broken line.

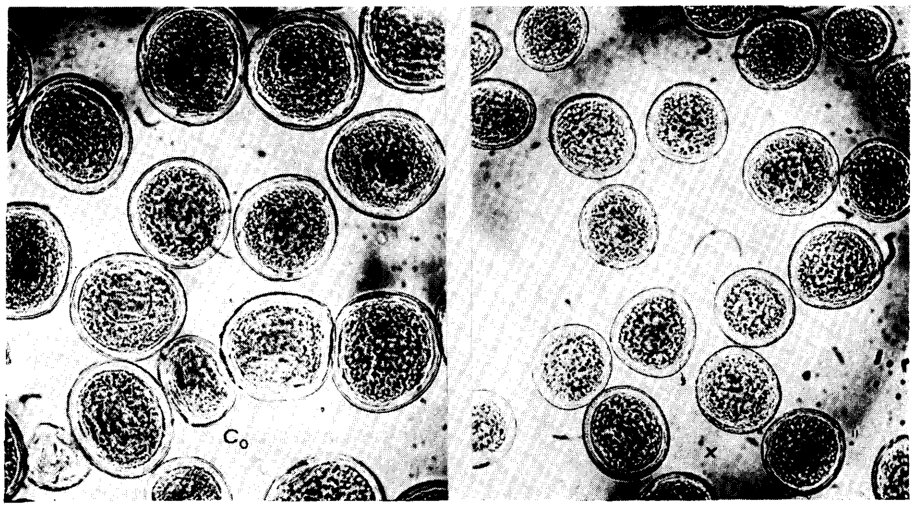


Fig. 8. — *Larix sibirica,* pollen grains of C_0, left, and 2x-control, right, x 160.

Table 2. — The outcome of crosses diploid x C_0 in pines

Species	Number of infl.		Number of seeds		% filled seeds	Seeds per cone	Number of C_1-seedlings	2n for C_1		
	Polli-nated	harves-ted	filled	empty				2x	3x	?
silv.	197	58	61	1,007	5.7	18.4	42	33	0	9
cont.	89	31	15	489	3.0	16.3	14	5	6	3

is fairly normal, at least for *P. silvestris,* and indicates that a normal number of archegonia has been fertilized — no fertilization not even empty seeds —. However, not less than 94.3% and 97.0% respectively of the seeds were empty, which means a very high mortality rate for zygotes and (or) embryos. Dissection of seeds has shown that this high frequency of empty seeds was established as early as in the beginning of July. The germinability of the filled seeds was good. In 33 of the 42 C_1-*silvestris* seedlings the 2n-number was stated to be 24, for 9 seedlings no counts could be made.

As for the origin of the diploid C_1-seedlings three possibilities can be pointed out:

1. contamination with foreign pollen
2. occurrence of diploid cells in the archesporogeneous tissue (mixoploidy)
3. tetraploid PMC:s might give rise to haploid grains in a low frequency.

Of the 14 C_1-*contorta* seedlings 5 were diploid, 6 triploid and for 3 counts are lacking. It is not fully established that all seedlings classified as triploids have three complete genomes *(Fig. 9).* In spite of all possible care, all the C_1-seedlings of *P. contorta,* the triploids and the diploids as well, died within two months of unknown causes. Of course, it is possible that the diploids, if they have originated from haploid pollen grains, produced by tetraploid PMC:s, have not possessed two complete genomes, and thus have had reduced viability.

Fig. 9. — *Pinus contorta,* C_1 radicula-mitosis, showing 66 chromosome arms, x 1,400.

In the spring of 1973, one C_0-tree of *Larix sibirica* gave a pollen quantity large enough for pollination of 33 female inflorescences on two diploid trees, but only 10 cones reached maturity, the rest being killed by late frost. These 10 cones gave 222 seeds, however, only one being filled and germinated, but the seedling died very soon. It is to be noted that *Larix* seeds develop to normal volume, also without pollination but then, of course they are empty.

The 18 female inflorescences, which occurred on C_0-*sibirica* in 1974 were all killed by late frost, a rather common event in our latitude. The larches flower in the beginning of April, when late frosts are frequent. In the spring of 1974, as low a temperature as —7.5° C was registered during the larch flowering.

C_0-*Picea Abies* has not yet produced enough pollen for pollination experiments. Most of the female inflorescences, which appeared in 1974, withered and dropped off already early in the summer. In the autumn only four cones remained, but their development had come to a premature standstill *(Fig. 10).* From these cones, 27 seeds of normal size could be extracted. However, all were empty. The inflorescences had not been pollinated artificially. The C_0-trees, however, are surrounded by a great number of diploid cones, which flowered abundantly and simultaneously, hence it can be safely postulated that the pollina-

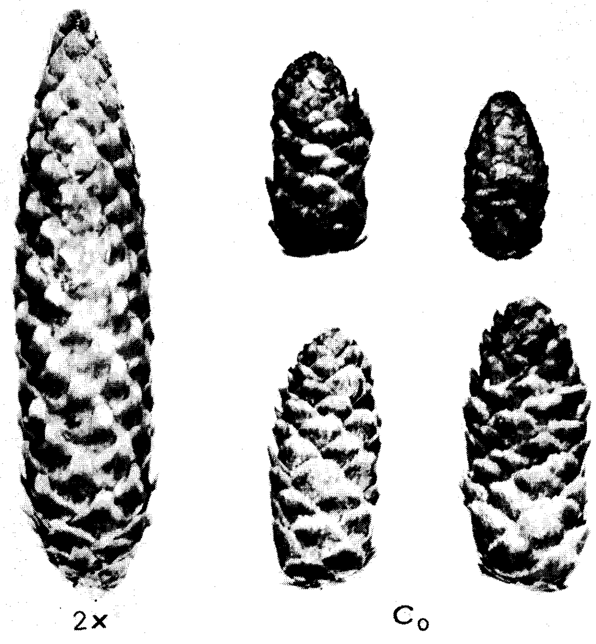


Fig. 10. — *Picea Abies,* underdevelop cones of C_0 in comparison with a normal sized diploid cone.

tion has been perfect. As a matter of fact the cones of *P. Abies* develop normally also without pollination. That the inflorescences in this case dropped off shortly after the flowering or gave rise to underdeveloped cones indicates that also disturbances in the cone ontogeny, i.e. diplontic sterility, is involved.

Discussion

Induction of polyploidy as a possibility for producing improved (more rapid growing) cultivars of forest trees has been put forward by several authors, as Nilsson-Ehle (1938), Schreiner (1938), Johnson (1939), Hyun (1953) and Gustafsson (1960), to mention only a few.

It has also been proved in some few broad-leaved species that artificially produced triploid populations actually have good growth. It is true for *Populus tremula* (Johnsson 1955), *Alnus glutinosa* (Johnsson 1949) and *Betula verrucosa* (Johnsson 1956). Triploid families of *Alnus glutinosa* tested up till now, however, are valueless on account of their tendency to crown break, and the triploids of *Betula verrucosa* grow well but no better than the diploids.

Concerning conifers, the expectations of obtaining gains in this way have been more restrained against a background where polyploidy has played a very limited role in the evolution of this group of plants, for instance, Eifler (1967). Nevertheless studies of the effects of polyploidy in conifers are under way here and there.

It has been clearly shown that C_0-conifers with strongly retarded growth and often also in other ways abnormal gradually start flowering. Thus flowering has occurred in C_0-*Pinus densiflora* at an age of 7 years (Zinnai 1953) and at the same age in C_0-*Larix* sp. (Illies 1957), in C_0-*Pinus rigida* at 9 years of age (Kim *et al.* 1967) and in C_0-*Pinus banksiana* before the age of 15 years (Kim and Lee 1973). We had to wait for almost 30 years for the first flowering in the C_0:s of *Pinus silvestris, Pinus contorta, Picea Abies* and *Larix sibirica,* reported in this paper. However, it is to be noted that *Pinus silvestris* and particularly *Picea Abies* are notoriously late flowering species, and also that the local climate is unfavourable for induction of flowering on account of the low summer temperature.

It is remarkable that only male flowering until now has appeared in *Pinus silvestris.* When young trees of this species start flowering, they normally produce only female flowers, the sex determination being physiological, the flower primordia on well-nourished twigs develop in a female direction and primordia on less well-nourished, over-shadowed twigs, in a male direction. On young trees, all twigs are well nourished with one-sided female flowering as a consequence. When, on the contrary, one-sided male flowering occurs on the C_0-trees, it is to be looked upon as a symptom of disturbed nutritive balance. In C_0-*Pinus banksiana,* however, female flowering has occurred (Kim and Lee 1973).

The pollen grains have shown similar deviations in *Pinus silvestris, P. contorta* and *Picea Abies,* consisting of supernumerary air sacs. The pollen germination in vitro is good.

Crosses diploid x C_0 in *Pinus silvestris* and *P. contorta* have given very low seed yields, which probably is to be ascribed to zygote- and (or) embryo lethality and which is in accordance with the statement of Kim and Lee (1973) that the seed of C_0-*P. banksiana* is "unfertile". Only the cross diploid x C_0-*P. contorta* has given some few triploid C_1-individuals, which, however, all died in the course of a couple of months.

The open pollinated female inflorescences of *Picea Abies* dropped off shortly after the flowering or the development of the cones had come to premature standstill and no germinable seeds were obtained.

The cross diploid x C_0-*Larix sibirica* gave only a single germinable seed, but the seedling died very early. The only known, sportaneous tetraploid tree of *Larix decidua,* produced no germinable seed (Christiansen 1950).

Illies (1966a), however, has registered germinability values of 8.3% to 17.9% for crosses C_0 x diploid *Larix sp.* and and of 32—33% for crosses in the reciprocal direction. Resulting tripolid C_1-individuals have shown a peculiar instability in somatic chromosome numbers, which have varied within the individuals, and cells with $2n = 2x$ and 4x have obtained increasing frequences. It means that disturbances occur at the mitotic divisions leading to unequal distribution of the chromosomes (Illies 1957, 1966a and b).

The fundamental requisite for the use of polyploidy in plant improvement is that crosses of the type diploid x tetraploid or reciprocal have so high fertility that mass-production of triploid populations is feasible. The experimental data, now present — meagre as they are — point in the opposite direction so far as the conifers are concerned. However, more information from enlarged materials must be gathered before a final evaluation of the possibilities of polyploidy in conifer improvement can be made.

Acknowledgements

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Summary

Flowering behaviour, pollen characteristics and fertility have been studied in C_0-individuals in *Pinus silvestris, P. contorta, Picea Abies* and *Larix sibirica,* when they started to flower at an age of about 30 years.

The tetraploidy, induced in the germinating seeds, is maintained up to this flowering age.

The pollen grains of the first-mentioned three species show a common abnormity, consisting in supernumerary air sacs. The C_0-pollen grains of *Larix sibirica* are considerably larger than the ones of diploid trees.

In no cases have viable, triploid offsprings been obtained, and it is concluded that the possibilities of polyploidy in conifer improvement seem doubtful at present on account of the sterility of the tetraploids. However, more information is needed before a final evaluation can be made.

Key words: Conifers, induced polyploidy, C-treatment, mixopolyploidy, supernumerary air sacs, pollen grain size, fertility, triploidy.

Zusammenfassung

In den Jahren 1941 bis 1945 mit Colchizin behandelte Individuen von *Pinus sylvestris* L., *Pinus contorta* Dougl. ex Loud., *Picea abies* (L.) Karst. und *Larix sibirica* (Muenchh.) Ledeb. wurden nach nunmehr fast 30 Jahren, d. h. nach Einsetzen der Blüte auf Blütenverhältnisse, Pollenbeschaffenheit und Fertilität untersucht. Hierbei stellte sich heraus, daß die in den damals keimenden Samen induzierte Tetraploidie bis zum Alter von rund 30 Jahren beibehalten wird. Die Pollenkörner von *Pinus sylvestris, P. contorta* und *Picea abies* zeigen z. T. überzählige Luftsäcke. Die Pollenkörner von *Larix sibirica* sind wesentlich größer als bei normalen diploiden Bäumen. Lebensfähige triploide Nachkommen konnten nicht erzielt werden. Daraus wird gefolgert, daß es nach dem gegenwärtigen Stand des Wis-

sens fraglich erscheint, die Polyploidisierung als nutzbringende Methode in der Nadelbaumzüchtung zu verwenden. Eine endgültige Beurteilung ist jedoch noch nicht möglich.

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Comparative Karyotype Analysis of Douglas-Fir

Pseudotsuga menziesii (Mirb.) Franco

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Introduction

Recent reports on the variation of nuclear volume and DNA content between genera and among species within genera in higher plants (13, 14, 16, 18, 24, 29, 31) may appear to contradict the DNA constancy theory put forward by Bovin *et al.* (1948) and Mirsky and Ris (1949).

Nuclear volume has been reported to vary with latitude; plants from northern latitudes tend to have a larger nuclear volume than plants of the same species from southern regions (8, 33, 34). The existence of intraspecific clinal variation in DNA content from south to north (13, 25, 26) has been reported. Stebbins (1964, 1966) indicated that the variation in nuclear characteristics, such as nuclear volume and DNA content, is not random, but has an adaptive significance.

The present study was undertaken on a similar but limited geographical basis to the investigation made by El-Lakany and Sziklai (1971) on intraspecific variation in DNA content, and compares this variation with karyotypic descriptions based on morphological characteristics of the chromosomes, De-Vescovi (1974).

Materials and Methods

Cone samples were collected from their natural habitat in 1966 and 1968 by the International Union of Forest Research Organization (IUFRO), and the seeds were extracted, cleaned and stored as described by Yao (1971).

One hundred and fifty open pollinated seeds from each of the four provenances (Covelo #1, Gasquet #2, Forks #3 and Spokane #4) *(Fig. 1)* were germinated on the Jacobsen Germinator. Some root-tips of all germinants, approximately 5 mm. long, were treated in monobromonaphthalene for three hours at room temperature, fixed in Farmer's solution, hydrolyzed in 1N HCl at 60° C for 10 minutes, stained in leuco-basic fuchsin for 60—90 minutes and then squased in 45% acetic acid. Each of the four locations provided approximately 120 metaphase plates with well spread chromosomes, of which three cells with clearly defined details were measured and analysed (9).

Numerous photomicrographs were taken from each cell to obtain prints of the chromosomes in sharp focus. These prints were then used to measure the chromosomes. Once all the chromosomes were measured on the prints, the short arm length (p), the long arm length (q), the total length (T), and the width (w), were calculated.

Arm ratio was based on long arm length (q) over short arm length (p). Centromere index (C.I.) was calculated using the formula (35) $\text{C.I.} = \frac{100p}{(p + q)}$ Relative chromosome length was calculated in relation to the longest individual chromosome. The maximum value was 1, assigned to the longest chromosome.

Morphological index (M.I.) was used as in a previous work by Gianelli and Howlett (1967). $\text{M.I.} = (p/q)\ (p + q)$.

The average width and the total length of the chromosomes were used for calculating the volume and it was assumed (4, 6, 22, 29, 36) that the cross section of the chromosome is a circle.

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