when it had a radicle of approximately 2 mm. in length. On December 20th, when germination was completed, 78 germinated seeds from each pollination, with the exception of Tree 3 × Self, which had only 52, were planted in individual pots in a greenhouse and raised under an 18- to 20-hour photoperiod. The pots were arranged in a randomized row design (13 pots/row) in six blocks. The Petri dishes, containing the remaining seeds, were opened and placed on a bench in a greenhouse. All the seedlings were examined periodically and any phenodeviants recorded.

On January 25th, 1963, measurements of hypocotyl length, number of cotyledons and mortality were recorded for the potted seedlings of the four complete blocks.

White Pine

In the spring of 1960, as part of the breeding program of the Ontario Department of Lands and Forests, a number of pollinations were made on grafts of 13 trees, whose ortets are located in a plantation at Pointe Platon, Quebec. A single tree of the same origin was used as male parent in all the pollinations, one of which was a self-pollination.

Cones from these pollinations were collected in the fall of 1961. Seeds were extracted and the full and empty seeds separated as previously described. Seed coats were removed and the seeds germinated on granitic sand in Petri dishes in December, 1961 (for method see FOWLER, 1959). The germinated seeds were planted in individual pots in a greenhouse and raised under an 18- to 20-hour photoperiod. When the seedlings were approximately six months old and had formed terminal buds, the total height and the length of the longest needle were measured on up to 20 seedlings from each pollination. These seedlings were ranked from 0 to 4 on the basis of foliage colour (0 = white, 4 = dark green). The seedlings were examined periodically and all abnormal seedlings were recorded.

Results

Jack Pine

The results of the pollination study are summarized in *Table IV - 1.*

Analysis of variance revealed the differences between trees to be highly significant for all factors studied except for cone set, percentage of set cones that matured, and percent germination. Many, if not most, of these differences probably have some relationship to genetic differences between these five trees, but as the trees were not replicated, it is not possible to separate differences caused by genetic variation from those caused by variation of the environments in which these trees were growing.

No significant differences were found between the effects of self- and cross-pollination on the number of cones set, the proportion of set cones that matured, cone length, weight of full seed and percent germination. The analysis revealed no significant differences in the number of seeds per cone developed after self- or cross-pollination, but the interaction between trees and pollens was highly significant. Trees 1 and 2 produced more numerous seed per cone from selfing than from crossing, while in the remaining three trees the reverse was true.

Self-pollination resulted in a sizeable reduction in the proportion of full seed. The average percentage of empty seed was 63.2 after self-pollination, compared with 11.3 after cross-pollination. The difference in the proportion of empty seeds after self- and cross-pollination of Tree 2 was only 5.5 percent. On the basis of number of full seeds produced per cone, this tree actually produced more full seeds after selfing (36.3) than after crossing (24.7). None of the seeds which floated in absolute ethyl alcohol contained living gametophyte tissue.

Germination of all seed lots was good. Over 95 percent of the seeds germinated on the fourth day after they were moved into the laboratory and germination was complete after six days. No differences were observed in rate of germination between trees and between pollens. Self-pollination yielded seeds whose germination was slightly poorer than that of cross-pollinated seed, but this difference was not significant. Thirteen cases of reverse germination were recorded. In reverse germination (ILLIES, 1952) the embryo is reversed in relation to the micropyle. The cotyledon-bearing tip of the embryo emerges from the micropylar end of the seed, while the radicle remains enclosed in the gametophyte tissue. Of the 13 seeds with reverse germination, two resulted from cross-pollination and 11 from self-pollination. Eight of these 11 seeds were found among the progeny of Tree 5. None of the cross-pollinated seed of this tree germinated abnormally. Analysis of germination percent, on the basis of normal emergence, revealed

Table IV — 1. — Jack Pine, *Pinus banksiana*, controlled pollinations.

Tree No.	Pollen	No. Ovulate Strobili	Cone Set Percent	Cones Matured Percent	Cone Length cm.	No. Seeds/ Cone	Empty Seed Percent	Seed Wt. mg.	No. Seeds to Germ.	Germ. Percent³)	Normal Germ. Percent	Survival Percent	Hypocotyl Length mm.	No. cotyledons
							Summary							
1	Self	31	68	95	36	44	76	4	173	97	96	78	31	4.5
	Cross	22	86	68	35	21	12	4	200	100	100	100	35	4.5
2	Self	29	79	87	53	49	26	5	200	96	96	100	33	4.9
	Cross	27	93	68	50	31	20	6	200	98	97	100	38	5.0
3	Self	15	93	71	46	32	84	5	53	98	98	77	38	5.1
	Cross	20	65	100	45	46	16	5	200	99	99	100	38	4.6
4	Self	31	52	69	49	46	50	5	239	99	99	96	36	4.7
	Cross	28	57	51	48	52	3	6	200	99	99	98	38	4.5
5	Self	30	100	97	41	39	80	4	215	96	92	98	39	4.8
	Cross	31	68	81	40	50	6	4	200	98	98	98	38	4.4
$\bar{x}$	Self		78	84	45	42	63	5		97	96	72	35	4.8
	Cross		74	74	44	40	11	5		99	99	99	37	4.6
							Analysis							
Trees			NS	NS	**	**	**	**		NS	**	**	**	**
Pollens			NS	NS	NS	NS	**	NS		NS	**	**	**	*
Trees × Pollens			—	—	NS	**	**	NS		NS	NS	**	**	NS

³) Includes all seeds from which viable embryos emerged (normal + reverse + double).

a highly significant difference between self- and cross-pollination.

When six weeks old, the seedlings resulting from self-pollination had shorter hypocotyls and a higher mortality than those from cross-pollination. These differences were highly significant, but the interaction between the influence of trees and pollens was also highly significant. The seedlings resulting from self-pollination of Tree 5 consistently had longer hypocotyls than the seedlings from cross-pollination. The interaction of trees and pollens in the analysis of survival resulted because almost all the mortality occurred among the self-pollinated progenies of Trees 1 and 3.

Seedlings from self-pollination had slightly more numerous cotyledons than those from cross-pollination. This difference was significant at the five percent level ($F = 4.41$, $F_{.05} = 4.21$).

Deviant seedlings were present among the self-pollinated progenies of Trees 1, 2 and 4 *(Figure IV — 1).*

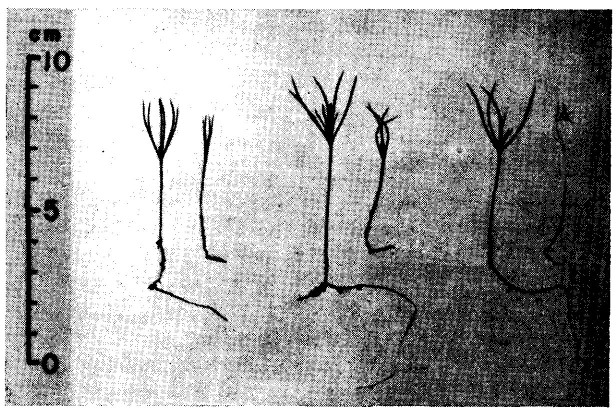

Figure IV — 1. — Normal and deviant jack pine seedlings obtained from self-pollination. — Left to right: 1 normal, 1 deviant; 2 normal, 2 deviant; 4 normal, 4 deviant.

Tree 1 × Self: Among the 135 seedlings of this pollination surviving to an age of about four weeks, 29 were small, chlorotic and with short cotyledons, which usually numbered four. The deviant seedlings had all withered and died by the sixth week. The seedlings were all normal in appearance when they first emerged and it was not until they were three to four weeks old that the deviant seedlings were readily distinguishable from their normal sibs. The ratio of normal to deviant seedlings was 3.7 : 1; however, mortality was high in this population and, as a high proportion of the deviant seedlings probably died, the ratio was likely closer to 3 : 1. The abnormalities were likely caused by a single recessive gene which was lethal in the homozygous condition. The seedlings, although normal in appearance when they first emerge, did not develop once their gametophyte food supply was exhausted.

Tree 2 × Self: Within a few days to a week after germination, the deviant seedlings of this pollination were readily distinguishable from their normal sibs. They were pale yellow in appearance at one to two weeks of age but, by the end of the fourth week, they were only slightly chlorotic. The two kinds of seedlings were no longer distinguishable by the sixth week. Of 192 seedlings surviving to two weeks, 175 were normal and 17 were chlorotic, a ratio of 10.3 : 1. Any explanation of the genetic basis for this segregation is purely conjectural. It is possible that this is based on a single recessive mutation in which a high proportion of homozygous seedlings die during the pro-embryo or embryo stages.

Tree 4 × Self: The deviant seedlings of this pollination, like those of Tree 2 × Self, were readily distinguishable from normal seedlings within a few days to a week after germination. At first, they had the appearance of albinos in that they lacked any green pigment. When these seedlings were about three weeks old, their cotyledons began to develop green pigments. Tissues developing from the apical growing point (stem and primary needles) were normal green in colour. The deviant seedlings were still distinguishable from their normal sibs after six weeks of age but it was evident that they would soon be indistinguishable. Fifteen seedlings of this type were found among 239 seedlings of this progeny which survived to an age of two weeks. This gave a ratio of 18.8 : 1 of normal to deviant which was close to the 15 : 1 ratio expected from selfing, when two heterozygous genes are involved. Under greenhouse conditions, these mutants survived as well as their normal sibs.

White Pine

The white pine pollinations were not designed to study the effects of selfing in this species. The data, showing the averages of the self-pollinated materials, as well as the range of averages and averages of the cross-pollinated materials, are presented in *Table IV - 2.* Eight of the 12 pollinations produced 18 or more seedlings which survived to an age of six months. One pollination failed to produce any cones and the three remaining pollinations produced only one, two and five seedlings. The ten crosses which produced seed were used to compute the range of averages and averages for cone set, number of seeds per cone and germination percentage, but only the seven crosses which produced 18 or more seedlings were used to obtain the range of averages and averages of the seedling characters.

Table IV — 2. — Comparison of averages for self- and cross-pollinated white pine.

	Full Seed/ Cone	Full Seed Percent	Germination Percent	Total Height cm.
Self Average	19.9	60.3	91.9	6.5
Cross Range	3.0—46.5	20.5—85.0	4.1—100.0	6.5—8.7
Cross Average	16.0	71.1	78.7	7.6

	Needle Length cm.	Foliage Colour	Twisted Needles Percent	Forked or Branching Percent
Self Average	6.5	2.9	15.3	12 0
Cross Range	5.7—9.2	3.3—3.9	0	0—5.7
Cross Average	8.2	3.6	0	2.9

The percent full seed, number of full seeds per cone and percent germination of seeds from the one selfing were well within the range of averages of those from the ten cross-pollinations. The shortest progeny resulting from cross-pollination and the progeny resulting from self-pollination had the same average height at six months of age. The needle length of the progeny resulting from self-pollination was well within the range of the averages of the other progenies. The foliage colour of the seedlings obtained from self-pollination was paler than that of any of the other progenies. The clearest and most reliable difference between the progenies obtained from self- and cross-pollination was in the presence or absence of two phenodeviant types. Twisted needles were observed in 15.3 percent of the seedlings obtained from self-pollination, while none of the seedlings among progenies from cross-pollination exhibited this character. The self-pollination also produced 12 percent seedlings with a lack of apical dominance as expressed by forking or branching. One of the progenies resulting from cross-pollination had 5.7 percent forked or branched seedlings while this character was very rare among the other progenies.

Discussion and Conclusions

Self-pollination clearly yielded results inferior to cross-pollination in all five of the jack pine trees studied, although the effects of selfing were expressed in different ways. Selfing produced fewer full seed in four trees, abnormal germination in seeds of one tree, seedlings with lower survival in two trees, with short hypocotyls in three trees, and with visible mutations in three trees.

The one white pine self-pollination yielded a progeny with some seedlings exhibiting two deviant types. In general, this progeny was more chlorotic and slower growing than the other progenies with which it was compared.

The five jack pines and the one white pine were somewhat self-fertile and self-compatible in that they produced at least some normal seedlings following self-pollination. This indicates that self-sterility in these species is governed by a relatively primitive lethal-semi-lethal gene system rather than by a system of incompatibility and sterility genes found in some more advanced genera. These results agree with those obtained from pollination studies of red pine and are likely to be true of other coniferous species. In essence, the more numerous the deleterious genes, or mutations a coniferous tree carries, the more self-sterile and self-incompatible it will be.

The effects of self-pollination in red pine were studied by FOWLER (1965a). In all, self-pollinations were successful on 46 trees. Not one of the progenies of these trees exhibited the effects of inbreeding to nearly as high a degree as any of the five jack pine and the single white pine studied. All three of these species are native to the most recently glaciated part of eastern North America. The three species were undoubtedly forced into warmer areas by the advancing ice sheets and re-occupied their northern areas as the ice sheets receded. It is not known whether they survived the glacial period in a common area or areas.

Pollen studies in which the three pine species are separated on the basis of pollen size (CAIN and CAIN, 1948; DAVIS, 1958 and OGDEN, 1959) indicate that they occupied the same general areas following glaciation. There is no evidence suggesting that the range of red pine was more restricted during the glacial period than the ranges of the other two species.

The main difference among these species, that could conceivably have a major effect on their genetic variability, is in their natural regeneration. It is also possible that differences in mutation rates may be important.

In trees, as in any other living organism, it is the breeding population and not the population as a whole that determines the genetic variability. Red pine is an intolerant species which is dependent upon disturbances, *e. g.* fire, to prepare the area for reproduction. In the course of these disturbances, many, if not most of the mature trees, are lost and the area is regenerated by a few surviving individuals. The reduction of the population size results in inbred populations in which there is a rapid elimination of recessive deleterious genes. The number of such deleterious genes that a self-pollinating (or inbred) population can carry in a heterozygous condition is much smaller than that in an outbreeding population.

Jack pine, like red pine, is an intolerant species, dependent upon disturbances to enable it to reproduce. Unlike red pine, the breeding population may be large. Almost all mature trees in an area can contribute to the regenerating population, even though they may not survive the disturbance which made regeneration possible. Seed formed prior to the disturbance, when conditions were favourable for cross-pollination, is available from serotinous cones, in which viable seed is stored for many years (RUDOLF, 1948).

Reproduction of white pine is not controlled by major forest disturbances to the same degree as of red pine and jack pine. In white pine, as in red pine, the surviving trees form the breeding population. The breeding population usually is not limited numerically to the same extent as red pine. One reason for this is that white pine is often found in areas where disturbances, such as fire, are not as pronounced, *e. g.* in hardwood associations.

Multiple fertilization is common in *Pinus* species. McWILLIAM (1959) reported that several pro-embryos are usually formed in *Pinus nigra* ARN. SARVAS (1962) found that *Pinus silvestris L.* averages 2.1 fertilizations per ovule. No estimates are available of the number of fertilizations in the ovules of red pine, jack pine and white pine. FOWLER (1959) reported finding up to seven immature embryos in white pine seed from northern Quebec. Usually only one embryo develops per ovule (BUCHHOLZ, 1959; McWILLIAM, 1959; SARVAS, 1962). Multiple fertilization undoubtedly reduces the frequency of ovule abortion, resulting from zygote, pro-embryo or embryo mortality.

If it is assumed, as is done by SARVAS (1962), that ovule abortion and, in turn, empty seeds result from recessive lethal (or semi-lethal) genes in a homozygous condition, multiple fertilization would greatly reduce the proportion of empty seeds resulting from this cause. *Table IV - 3* shows the reduction of aborted ovules or empty seeds expected in a self-pollinated tree, heterozygous for one recessive lethal gene, as the number of fertilizations within a single ovule increases.

Table IV — 3. — Expected percentage of aborted ovules in a self-pollinated tree heterozygous for one recessive lethal gene.

Number of fertilizations per ovule	Expected percentages of aborted ovules — empty seeds
1	25.00
2	6.25
3	1.56
4	.39
5	.01

If it is assumed that all ovule abortion is caused by recessive deleterious genes in a homozygous condition, that no linkage takes place and that the number of fertilizations per ovule is known, an estimate of the minimum number of recessive deleterious genes carried by a tree can be obtained from the proportion of hollow seeds produced after self-pollination. This is only an estimate of the number of deleterious genes that affect the developing organism during the period from fertilization to completion of seed development.

On the average, the five jack pine trees studied produced 63 percent empty seeds. Assuming that two fertilizations occur per ovule and that the deleterious genes are all lethal when in a homozygous condition, the average minimum number of alleles for which these trees are heterozygous is 15. As many of such deleterious genes are probably semi-lethal and as at least some of them must be linked, the actual number is undoubtedly much larger. If more than two fertilizations occur per ovule, the estimate must also be increased. When the whole life cycle of a tree is considered, the number of deleterious genes that a tree may carry could conceivably be in the hundreds or even thousands.

Despite the inadequacies of the preceding estimates, it is evident that the five jack pine trees studied (and probably jack pine in general) are heterozygous for a large number of deleterious alleles.

The evidence available from inbreeding studies is limited for white pine. The work of JOHNSON (1945) and the brief study reported in this paper did not reveal any differences in the proportion of full or empty seed from self- and cross-pollination in white pine. This may indicate that white pine does not carry the same high proportion of recessive deleterious genes as jack pine. This could also be the result of a higher number of fertilizations per ovule. Inbreeding depression, as expressed by reduced growth, was reported for white pine by JOHNSON (1945), PATTON and RIKER (1958), and is supported by this study.

It would appear that deviant seedlings are not uncommon in white pine progenies resulting from selfing. Two such deviants occurred among the progeny obtained from self-pollination of the single tree used in this study and JOHNSON (1945) reported deviant seedlings among the progeny of another self-pollinated tree.

It is not possible from the available information to determine exactly how white pine compares with red pine and jack pine in respect to genetic variability. It undoubtedly contains more genetic variability, as expressed by the number of recessive mutant genes which it carries, than red pine and probably less than jack pine.

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Summary

The effects of self-pollination on cone set and the percentage of set cones matured and on cone, seed and seedling characters were studied on five jack pines and one white pine. All of these trees were found to be partially self-fertile. In the five jack pine trees examined, self-pollination resulted in a reduced number of full seed in four trees, abnormal seed germination in one tree, lower survival of seedlings in two trees, seedlings with short hypocotyls in three trees and seedlings with deviant types in three trees. The one self-pollinated white pine produced a progeny which was more chlorotic and slower growing than progenies from cross-pollinated white pines. The progeny resulting from self-pollination contained seedlings of two deviant types.

The relative differences between the results obtained from self-pollination of jack and white pine and those found previously in red pine are discussed.

The genetic variability exhibited by the three species is discussed in relation to their method of natural reproduction. The normally large breeding population in jack pine enables this species to maintain a high degree of genetic variability, while the small breeding populations common in red pine probably limit genetic variabilty.

Jack pine, as a species, is more heterozygous than red pine. White pine is probably less heterozygous than jack pine and more heterozygous than red pine, at least in respect to seedling characteristics.

Résumé

Titre de l'article: *Autofécondation chez Pinus resinosa Ait. — IV. Comparaison avec d'autres pins du nord-est de l'Amérique du Nord.*

On a étudié sur cinq *Pinus banksiana* et un *Pinus strobus* les effets de l'autofécondation sur les caractères suivants: production de cônes, pourcentage de cônes mûrs, caractères des cônes, des graines et des semis. Tous ces arbres sont partiellement autofertiles. En ce qui concerne les *Pinus banksiana*, on a obtenu une réduction du nombre de graines pleines pour quatre arbres, une germination anormale pour un arbre, une faible survie des semis pour deux arbres, des semis avec des hypocotyles courts pour trois arbres et des semis avec des formes anormales pour trois arbres. Le *Pinus strobus* autofécondé a produit des descendants plus chlorotiques et moins vigoureux que ceux issus de pollinisation croisée. Les descendants autofécondés comprenaient des semis anormaux de deux formes différentes.

La discussion porte sur les différences constatées dans les résultats d'autofécondation de ces deux espèces comparés avec ceux obtenus précédemment pour *Pinus resinosa*. La variabilité génétique exprimée par les trois espèces est étudiée en fonction de leur mode de reproduction naturelle. Le large effectif des populations de *Pinus banksiana* permet à cette espèce de maintenir un haut degré de variabilité génétique tandis que le faible effectif des populations de *Pinus resinosa* limite probablement cette variété.

Pinus banksiana, en tant qu'espèce, est plus hétérozygote que *Pinus resinosa*. *Pinus strobus* est probablement moins hétérozygote que *Pinus banksiana* et plus que *Pinus resinosa* au moins en ce qui concerne les caractéristiques des semis.

Zusammenfassung

Titel der Arbeit: *Effekte der Inzüchtung bei Pinus resinosa Ait. — IV. Vergleiche mit anderen Pinus-Species des Nordostens.*

Die Effekte der Selbstung auf den Zapfenansatz und den Prozentsatz an ausgereiften Zapfen, ferner auf Zapfen-, Samen- und Sämlingsmerkmale wurden bei 5 Bankskiefern und bei einer Strobe untersucht. Alle diese Bäume zeigten, daß sie partiell selbstfertil waren. Von den 5 Bankskiefern reduzierten 4 Bäume nach der Selbstung die Zahl der vollen Samen; anomale Samenkeimung erfolgte bei einem Baum, eine geringere Anzahl an überlebenden Sämlingen fand man bei 2 Bäumen, und abweichende Sämlingstypen ergaben sich bei 3 Bäumen. Die einzige Selbstung bei der Strobe ergab eine Nachkommenschaft, die chlorotischer und geringwüchsiger war als Kreuzungsnachkommenschaften. Diese Selbstungsnachkommenschaft enthielt auch 2 abweichende Sämlingstypen.

Unterschiede zwischen den Ergebnissen der Selbstung bei der Bankskiefer und der Strobe und den Selbstungen bei *Pinus resinosa* wurden besprochen.

Die den 3 Species eigene genetische Variabilität wird im Zusammenhang mit ihrer natürlichen Reproduktionsweise diskutiert. Die normalerweise umfangreicheren Populationen der Bankskiefer befähigen diese, einen hohen Grad an genetischer Variabilität zu erhalten, während die im allgemeinen kleinen Bestandespopulationen bei *Pinus resinosa* wahrscheinlich deren genetische Variabilität einschränken.

Die Bankskiefer als Art ist heterozygoter als *Pinus resinosa.* Die Strobe ist wahrscheinlich weniger heterozygot als die Bankskiefer und heterozygoter als *Pinus resinosa*, wenigstens hinsichtlich ihrer Sämlingsmerkmale.

Literature Cited

Arend, J. L., Smith, N. F., Spurr, S. H., and Wright J. W.: Jack pine geographic variation, five-year results from lower Michigan tests. Mich. Acad. Sci. 46: 219—238 (1961). — Batzer, H. O.: Jack pine from Lake States seed sources differ in susceptibility to attack by the white pine weevil. Lake States For. Expt. Sta., U. S. Forest Serv., Tech. Note 595, 2p. (1961). — Buchholz, J. T.: The pine embryo and the embryos of related genera. Trans. Illinois State Acad. Sci. 23: 117—125 (1931). — Cain, S. A., and Cain, L. G.: Palynological studies at Sodon Lake. II size-frequency studies of pine pollen, fossil and modern. Am. J. Botany 35: 585—591 (1948). — Davis, M. B.: Three pollen diagrams from central Massachusetts. Am. J. Sci. 256: 540—570 (1958). — Fowler, D. P.: Rapid germination of white pine seed. For. Chron. 35: 203—211 (1959). — Fowler, D. P.: Effects of inbreeding in red pine, *Pinus resinosa* Ait. I. Natural variation. Silvae Genetica 13: 170—177 (1964). — Fowler, D. P.: Effects of inbreeding in red pine, *Pinus resinosa* Ait. II. Controlled pollination studies. Silvae Genetica 14: 12—23 (1965 a). — Fowler, D. P.: Effects of inbreeding in red pine, *Pinus resinosa* Ait. III. Factors affecting natural selfing. Silvae Genetica 14: in press (1965 b). — Giertych, M. M., and Farrar, J. L.: The effect of photoperiod and nitrogen on the growth and development of seedlings of jack pine. Canadian J. Botany 39: 1247—1254 (1961). — Giertych, M. M., and Farrar, J. L.: A provenance study of jack pine seedlings. Silvae Genetica 11: 111—114 (1962). — Illies, Z. M.: Keimlingsabnormitäten bei *Picea abies* (L) Karst. Z. Forstgen. Forstpflanz. 2: 28—32 (1952). — Johnson, L. P. V.: Reduced vigor, chlorophyll deficiency and other effects of self-fertilization in *Pinus.* Canadian J. Res. C. 23: 145—149 (1945). — McWilliam, J. R.: Interspecific incompatibility in *Pinus.* Am. J. Botany 46: 425—433 (1959). — Ogden, J. G.: A late-glacial pollen sequence from Martha's Vineyard, Massachusetts. Am. J. Sci. 257: 366—381 (1959). — Patton, R. F., and Riker, A. J.: Blister rust resistance on eastern white pine. Proc. 5th Northeast. For. Tree Improvement Conf. 1957: 46—51 (1958). — Pauley, S. S., Spurr, S. H., and Whitmore, F. W.: Seed source trials of eastern white pine. Forest Sci. 1: 244—256 (1955). — Rudolf, P. O.: Silvical characteristics of jack pine *(Pinus banksiana).* Lake States For. Expt. Sta., U. S. Forest Serv., Sta. Paper 61. 31p. (1958). — Rudolph, T. D., Libby, W. J., and Pauley, S. S.: Jack pine variation and distribution in Minnesota. Minn. For. Note 58. 2p. (1957). — Santamour, F. S. Jr.: Seasonal growth in white pine seedlings from different provenances. Northeast. For. Expt. Sta.. U. S. Forest Service, Res. Note 105. 4p. (1960). — Sarvas, R.: Investigations on the flowering and seed crop of *Pinus silvestris.* Commun. Inst. Forestalis Fenn. 53: 198p. (1962). — Schantz-Hansen, T., and Jensen, R. A.: The effect of source of seed on growth of jack pine. J. For. 50: 539—544 (1952). — Vaartaja, O.: Evidence of photoperiodic ecotypes of trees. Ecol. Monographs 29: 91—111 (1959).

Changes of the Daily Rhythm of Mitosis in Pinus nigra Arn. Caused by Gamma Rays*)

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Introduction

On the occasion of our investigations into the chromosomes of the somatic cells of *Picea abies* Karst. (Bevilacqua and Vidaković, 1963) we noticed that it is not possible to find at any time of day a sufficient number of mitotic stages. Taking into consideration that in our further investigations into the mitoses and chromosomes of various coniferous species we had to obtain a larger number of cells in mitosis, we carried out investigations about the diurnal fluctuation of the mitotic frequence.

The subject of these investigations was the Austrian Pine. We made the measurements of the frequency of mitosis in the root tips of the seed which were irradiated with gamma rays of ^{60}Co. As controls nonirradiated material was used.

It should be emphasized that this work lays no claim to a quantitative study but only to a qualitative investigation for the purpose of guidance.

Material and Working Method

The seed we used for these investigations was supplied from Slovenia and collected in a natural stand (locality Divača-Pivka) in the autumn of 1961. The germinative capacity of the seed amounted to 90%; the irradiation of seeds with gamma rays was carried out in the spring of 1962 by means of a ^{60}Co-source of 350 curies. The radiation doses were 100, 1000 and 5000 r respectively while the humidity of seeds during the irradiation was 8.41%.

The seed germinated at room temperature in Petri dishes. On achieving a 3—4 mm. length the roots were cut and fixed in acetic alcohol and stored into a refrigerator at 4° C. Immediately before the making of slides the roots were macerated in 1 N HCl at 60° C for 45—60 minutes. Then they were stained during 30 minutes in aceto-carmine on the slides. On covering the slides with the cover slip they were slightly pressed and weakly warmed over a gas burner. By covering the rims of the coverslips with molten paraffin we made semipermanent mounts.

Experimental Work

I. Programme of experiments

From each sample of the irradiated material and from the controls were taken every hour five roots. From each root was made one slide. The total number of preparations was 480. Each of these preparations was examined separately. The examination was carried out with an enlargement of 60×, and this always under the same conditions, i. e. in each preparation always the same area was examined (a total of 35 horizontal rows; read from the vernier scale for vertical shifting this interval was 0.5 mm.).

Under the "prophase" we recorded only the advanced

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