Vorläufige Beobachtungen über die Vererbung von Nadel-Eigenschaften zeigen, daß F,-Artbastarde sich intermediär verhalten zwischen den Elternarten. Besonders bei quantitativen Merkmalen liegen sie bei der F, etwa in der Mitte zwischen den beiden sich unterscheidenden Nadeleigenschaften; bei anderen Eigenschaften können sie dem einen oder dem anderen Elter ähnlicher sein, obgleich such da einige Unterschiede nur gering sind.

Résumé

Titre de l'article: *Caractéristiques des aiguilles des pins hybrides.*

Les auteurs ont étudiés en 1953, 1956 et 1957 la morphologie et l'anatomie des aiguilles de 42 pins hybrides poussant à l'Institut de Génétique forestière de Placerville (Californie). Ces pins hybrides comprennent 35 hybrides interspécifiques de premiere generation de 30 espèces, 2 trihybrides, 3 croisements en retour et deux hybrides intraspecifiques ou variétaux. Tous sauf un sont des hybrides artificiels.

Les 18 tableaux dans lesquels ces hybrides sont comparés à leurs parents d'après les caractéristiques des aiguilles peuvent être utilisés pour l'identification des plants hybrides en particulier les jeunes plants sans cônes. I1 n'est cependant pas toujours possible d'identifier de façon certaine, d'après les seuls caractères des aiguilles, des hybrides entre quelques espèces tres voisines.

Les premières observations sur la transmission héréditaire des caracteres des aiguilles montrent que les hybrides interspécifiques de premiere génération sont intermédiaires entre les parents pour a peu pres la moitié des caractères nettement différenciés, en particulier les caracteres quantitatifs, et pour le reste des caractères, ressemblent plus a l'un ou a l'autre parent, bien que certaines différences soient assez faibles.

Literature Cited

(1) CALLAHAM, ROBERT Z.: Needle oils of three pine species and species hybrids. Forest Science 2: 101–105 (1956). — (2) COULTER, JOHN M., and Rose, J. N.: Synopsis of North American pines, based upon leaf-anatomy. Bot. Gaz. 11: 256–262, 302–309 (1886). — (3) CRITCHFIELD, WILLIAM B.: Geographic variation in Pinus *contorta.* Maria Moors Cabot Found. Pub. 3, 118 pp., 1957. — (4) DALLIMORE, W., and JACKSON, A. BRUCE: A handbook of Coniferae including Ginkqoaceae. Ed. 3, 682 pp., 1948. — (5) DOI, TOHEI, and MORIKAWA, KIN-ICHI: An anatomical study of the leaves of the genus Pinus. Kyushu Imp. Univ. Dept. Agr. Jour. 2: 149–198 (1929). — (6) DUFFIELD, J. W.: Relationships and species hybridization in the genus *Pinus.* Zeitschr. Forstgenetik 1: 93–97 (1952). — (7) DUFFIELD, J. W., and RIGHTER, F. I.: Annotated list of pine hybrids made at the Insitute of Forest Genetics. Calif. Forest and Range Expt. Sta. Forest Res. Notes 86, 9 pp., 1953. — (8) FERRÉ,, Y. DE: Les formes de jeunesse des Abiétacées ontogénie-phylogénie. Lab. Forest Toulouse Trav. Tome 2, vol. 3, art. 2, 277 pp., 1952. — (9) FOWLER, D. P., and HEIMBURGER, C.: The hybrid Pinus peuce GRISEB. × *Pinus* strobus L. Silvae Genetica 7: 81-86 (1958). — (10) HARLOW, W. M.: The identification of the pines of the United States, native and introduced, by needle structure. N. Y. State Col. Forestry Tech. Pub. 32, 21 pp., 1931. (Reprinted 1947.) — (11) HELMERS, AUSTIN E.: The ecological anatomy of ponderosa pine needles. Amer. Midland Nat. 29: 55–71 (1943). — (12) KOEHNE, EMIL: Deutsche Dendrologie. 601 pp., 1893. — (13) LITTLE, ELBERT L., JR.: Check list of native and naturalized trees of the United States (including Alaska). U. S. Dept. Agr., Agr. Handb. 41, 472 pp., 1953. — (14) MERGEN, FRANÇOIS: Genetic variation in needle characteristics of slash pine and in some of its hybrids. Silvae Genetica 7: 1–9 (1958). — (15) MERGEN, FRANÇOIS: Applicability of the distribution of stomates to verify pine hybrids. Silvae Genetica 8: 107–109 (1959). — (16) PILGER, R.: Pinaceae. In: ENGLER, A., and PRANTL, K.: Die Natürlichen Pflanzenfamilien. Ed. 2, 13: 271–342 (1926). — (17) RIGHTER, F. I., and DUFFIELD, J. W.: Interspecies hybrids in pines. Jour. Hered 42: 75–80 (1951). — (18) RIGHTER,, F. I., and DUFFIELD, J. W.: Hybrids between ponderosa and Apache pine. Jour. Forestry 49: 345–349 (1951). — (19) RIGHTER, F. I., and STOCKWELL, W. P.: Fertile speçies hybrid, Pinus murraybanksiana. Madroiio 10: 65-69 (1949). — (20) SCHÜTT, P., and HATTEMER, H. H.: Die Eignung von Merkmalen des Nadelquerschnitts für die Kiefern-Bastarddiagnose. Silvae Genetica 8: 93–99 (1959). — (21) SHAW, GEORGE R.: The genus Pinus. Arnold Arboretum Pub. 5: 96 pp. (1914). (Reprinted 1958.) — (22) STOCKWELL, PALMER, and RIGHTER, F. I.: *Pinus:* the fertile species hybrid between knobcone and Monterey pines. Madroiio 8: 157–160 (1946). — (23) STONE,' E. C., and DUFFIELD, J. W.: Hybrids of sugar pine by embryo culture. Jour Forestry 48: 200–201 (1950). — (24) SUTHERLAND, M.: A microscopical study of the structure of the leaves of the genus Pinus. New Zeal. Inst. Trans. Proc. 63: 517–568 (1934). — (25) VIDAKOVIĆ, M.: Investigations on the intermediate type between the Austrian and the Scots pine. Silvae Genetica 7: 12–49 (1958). — (26) WEIDMAN, R. H.: Trees in the Eddy Arboretum. Calif. Forest and Range Expt. Sta. Forest Res. Notes 53, 8 pp., 1947. — (27) ZOBEL, BRUCE: The natural hybrid between Coulter and Jeffrey pines. Evolution 5: 405–413 (1951).

Microsporogenesis in Abies[1])

By FRANÇOIS MERGEN and DONALD T. LESTER,

Yale University, School of Forestry, New Haven, Conn., U.S.A.

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Introduction

The genus ***Abies*** contains more than 40 species, and members of the genus are found in the northern hemisphere in a band that circumscribes our planet. On the American Continent its southern range extends into the mountains of Guatemala, and in Africa it occupies certain sites in Algeria. Many species are exploited commercially in forestry and planted trees are effectively used as ornamentals. Despite their wide distribution and commercial importance, members of this genus have received a minimum of attention from forest scientists. Little is known about the phylogenetic relationships within this genus, and past hybridization attempts have been sporadic and on too limited a scale to be of great help. There are, however, reports on natural hybrids, and several species have yielded hybrids from artificial crosses. Because of the neglect of this genus, there are good opportunities to contribute basic knowledge on reproductive habits, normal and abnormal cytology, as well as on variation patterns in individual species and hybrids. Various aspects of the genetics of ***Abies*** are currently being investigated, such as crossing patterns, karyotypes, and responses to mutagens. Results from treatment with colchicine have been prepared (MERGEN and LESTER, 1961).

There is no complete description of the phenolgy of flowering in ***Abies*** in either forestry or botany literature, but there have been reports on some of the later stages

[1]) This study was supported by a grant from the National Science Foundation for research in forest genetics (G-8891).

of microsporogenesis and embryogeny (HOFMEISTER, 1848; MIYAKE, 1903; and HUTCHINSON, 1914; and 1915). In these reports the various stages were illustrated by line drawings, and no photographs on microsporogenesis are available. Neither has the cytology of microsporogenesis been described in detail.

Two aspects of pollen formation in *Abies* are described in this report. *Part I* is a pictorial presentation of the phenology of male flower and pollen development, and *Part II* is a detailed description of the cytology of microsporogenesis. Successful controlled pollination is dependent upon a knowledge of the flower phenology of the species. Unless the experimenter knows his material, failures in obtaining hybrid seed are as much a reflection of his methods as of the genetic incompatibility barriers that might exist.

As a matter of convenience the term "male flower" is used throughout the study to refer to the microsporangiate strobilus.

Materials and Methods

The fir trees that were used in this study are growing in the George P. Brett Pinetum of Yale University at Fairfield, Connecticut, and at the time of the study were about 40 years old. Trees of four representative species ware selected: *Abies sachalinensis* MAST., *Abies homolepis* SIEB. and ZUCC., *Abies nobilis glauca* BEISS., and *Abies* × *Boresii regis* MATTF.[2]) Detailed observations on the course of microsporogenesis were limited to *Abies sachalinesis* and *Abies homolepis*. The first collection of branches bearing male flower buds for the detailed cytological study was made during December, while the collection for the pictorial study was started on January 27. Subsequent collections were made periodically until mature pollen was shed in May.

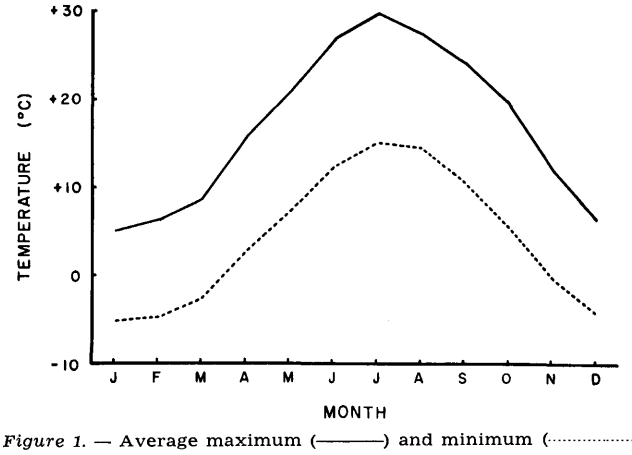

Figure 1. — Average maximum (———) and minimum (·····) temperatures for 1950—1954 for Norwalk, Connecticut.

In *Figure 1*, are presented the average maximum and minimum temperatures from an adjacent weather station, so that comparisons in stages of development can be made with trees growing in other regions.

For the pictorial presentation of the phenology of male flower development in *Figures 2, 3, 4 and 5*, four types of illustrations were prepared for each date of collection to illustrate (1) the outward appearance of the flower buds (2) the condition of the male strobilus devoid of scales, (3) a cross-section through the center of the bud, and (4) the condition of the developing microspores. To preserve the natural appearance, photographs for the first two types of illustrations were taken as soon as possible after the branches were brought in from the field. A metric ruler was placed in the macrophotographs to facilitate comparison at the various stages.

The scales were removed from several representative buds from each collection to facilitate the fixing, embedding and sectioning. Formalin-acetic acid-alcohol was used as a fixing agent, and the buds were embedded in Tissuemat. Longitudinal sections were cut on a rotary microtome at a thickness of 20—25 M, and these were stained in DELAFIELD'S hematoxylin and safranine. For the cytological observations of the developing microspores, several microsporophylls were crushed and the contents were fixed and stained in acetocarmine.

Results

Part I. Pictorial presentation of phenology of male flower and pollen development.

To obtain a good general picture of the male flower development, the results for each species are presented separately in a composite plate as follows: *Figure 2 = Abies sachalinesis; Figure 3 = Abies homolepis; Figure 4 = Abies nobilis glauca;* and *Figure 5 = Abies × Boresii regis.* The date of collection for each series of photographs is given.

In the *Abies* species under observation, the male strobili were borne on the lower side approximately midway between the terminal bud and the base of the branchlet of the current year's growth. They are found over a distance of three to ten cm. depending on the species, and the vigor of the branches. They become noticeable in the fall, and by mid-winter their presence is quite pronounced.

Abies sachalinensis: At the first collection on January 27, the length of the male flowers varied between three and four mm., and they were covered with a layer of whitish glaucous material. The cells within the microsporangia were in the primitive archesporial stage, with a pronounced tapetal layer surrounding them. By February 26, the strobili had enlarged considerably, and the archesporial cells had become differentiated into microspore mother cells. These typically rounded cells were filled with starch grains, and they remained in this condition for a period of about six weeks. The collections on April 8 revealed little outward changes with the exception of a slight enlargement, while the microspore mother cells had started the meiotic division. By April 12, well-formed microspores were present in most of the strobili. At this stage the strobili started to elongate rapidly, but the individual microsporophylls were still covered by the outer scales. The naked strobili had a dull green color in the lower part, and were purple toward the apex. From this date on, the external and internal condition changed rapidly, and by April 29, the microsporangia started to dehisce, liberating fully-formed pollen. At this stage the strobili were about 12 cm. long, and on three of the four trees available, they were bright yellow color, while on one tree they were reddish-brown.

Abies homolepis: At the start of the study the male strobili had a somewhat pointed apex and their over-all length was between three and four mm. Only moderate amounts of resin were present on the outer scales. The strobili without scales were almost circular in longitudinal sections and had

[2]) *Abies sachalinensis* and *Abies homolepis* grow naturally in northern Japan, the natural range of *Abies nobilis glauca* is in the western part of the United States, while *Abies* × *Boresii regis* is a hybrid between *Abies cephalonica* LOUD. and *Abies alba* MILL. that occurs naturally in Greece.

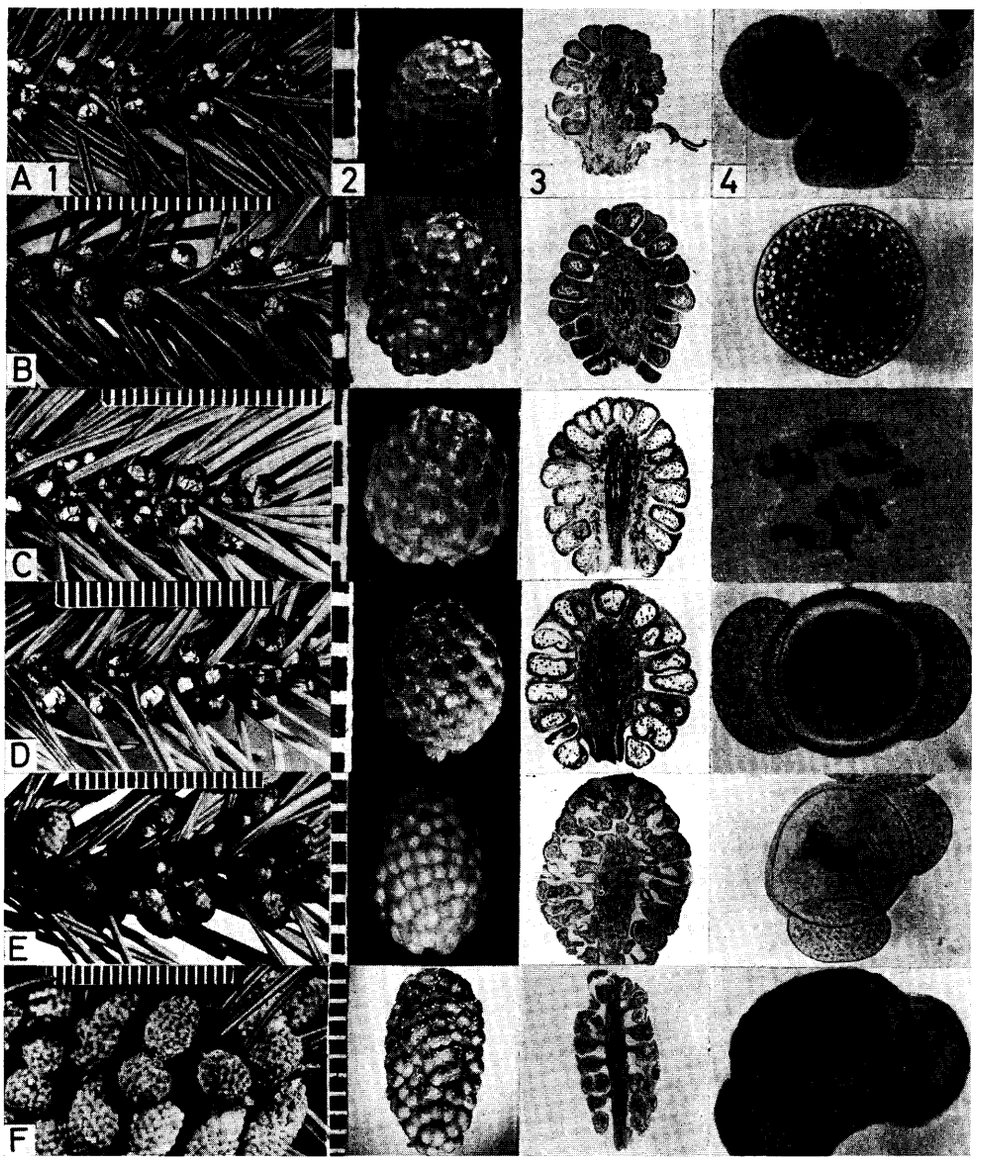

Figure 2. — Development of staminate flower buds in Abies sachalinensis. Scale in millimeters. — A1, branchlet; 2, flower bud; 3, longitudinal section of bud; 4, stage of microsporogenesis. — *Dates of collection:* A, January 27; B, February 26; C, April 3; D, April 14; E, April 19; and F, April 29. — *Stages of microsporogenesis:* A4, primitive archespore, 260×; B4, microspore mother cell, 380×; C4, diakinesis of meiotic prophase, 480×; D4, immature pollen grain, 350×; E4, second anaphase of immature pollen grain, 360×; F4, mature pollen grain at time of shedding, 250×.

a wide central axis. The cells within the microsporangia seemed homogeneous in the primitive archesporial stage. On April 6, the strobili had doubled their size but they had retained the rounded appearance. The cells within the microsporangia were separating, and the microspore mother cells had started meiosis. On April 19, fully formed microspores were present. The naked buds were either bright green or light yellow in color on the outside, while the color of the inner scales ranged from reddish-brown to lavender. By May 4, the strobili had ruptured the protective scales, the microspores had completed the second vegetative division, and terminal scales on the sporophylls were prominent. When mature on May 7, the strobili were yellow, had an oblong shape with marked terminal scales, and they had a total lenght of about 12 mm. Pollen started to dehisce freely on this date.

Abies nobilis glauca: During January, the male flowers were quite variable in size, some being as long as 8 mm. The outside scales were covered with a resinous material, and the terminal scales on the microsporophylls were pronounced. Primitive archesporial cells with well-defined nucleoli were present throughout. On April 6, the strobili had enlarged considerably, and the archesporial cells had differentiated into microspore mother cells. By April 19, the meiotic division was well in progress and cells in various stages of divison were present. The naked strobili were yellow-green with a purple apex. At this stage, irregularities were observed in the chromosome movement, the details of which are discussed in *Part II.* After this date, the strobili enlarged rapidly and the resinous layer ruptured and exposed the individual sporophylls. Microspores were present in buds collected on April 26, and by May 18 the microsporangia had dehisced and pollen grains were being shed. At this stage the strobili were about 18 mm. long, and 8 mm. wide with pronounced terminal scales on the microsporophylls. They were glaucous, and purple.

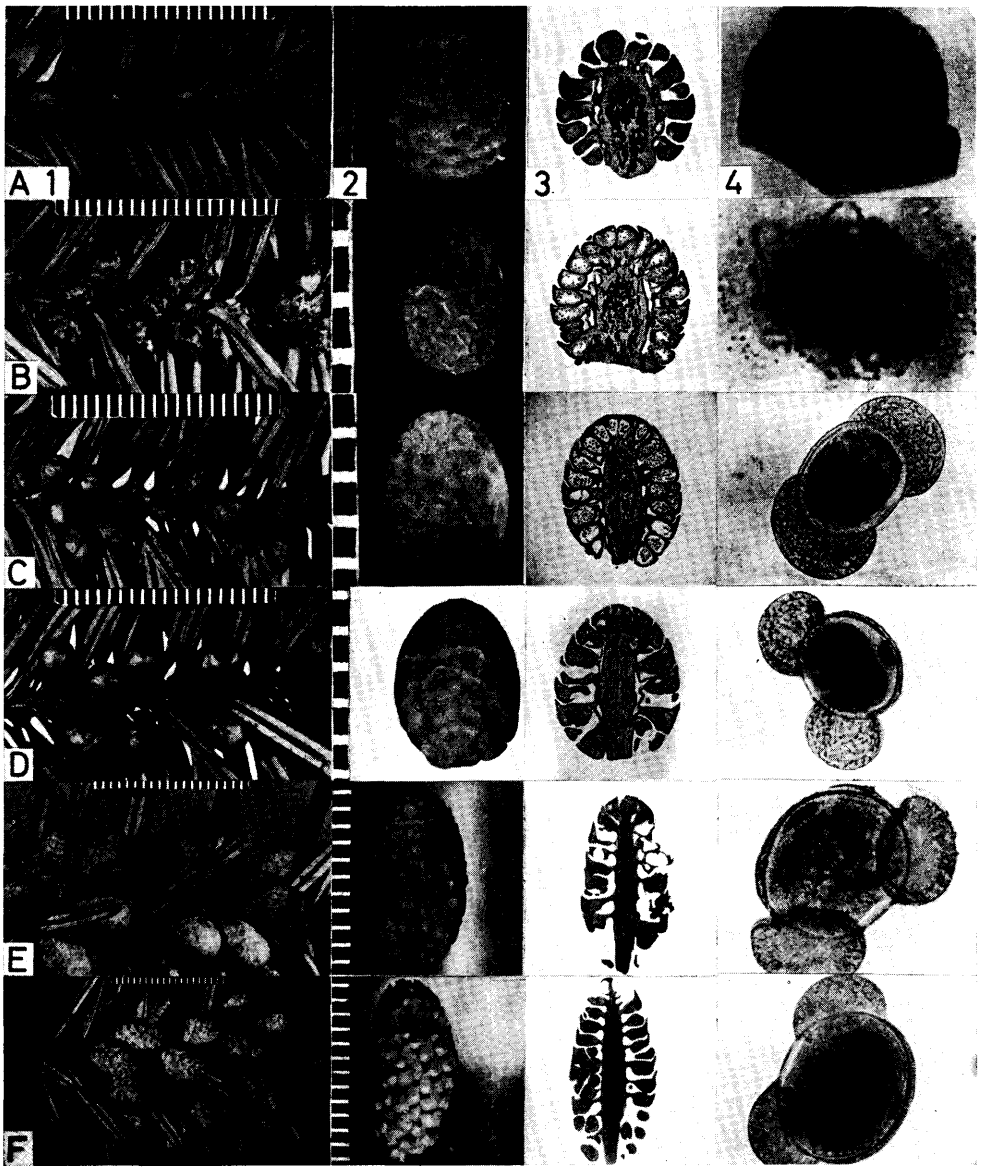

Figure 3. — Development of staminate flower buds in Abies homolepis. Scale in millimeters. — A1, branchlet; 2, flower bud; 3, longitudinal section of bud; 4, stage of microsporogenesis. — *Dates of collection:* A, January 27; B, April 8; C, April 19; D, April 26; E, May 4; and F, May 7. — *Stages of microsporogenesis:* A4, primitive archespore, 400×; B4, meiotic prophase nucleus of microspore mother cell, 670×; C4, immature pollen grain, 200×; D4, second interphase of immature pollen grain, 210×; E4, second metaphase of immature pollen grain, 240×; and F4, mature pollen grain at time of shedding, 180×.

Abies × *Boresii regis:* On January 27, the strobili had an average length of 5 mm., and the scales that covered the round interior structure formed a pointed apex. Little resin was present on these scales. The microsporangia were fully developed and were filled with angular primitive archesporial cells. By March 11, the microspore mother cells started the reduction division, and at this date the strobili still had a pointed apex. Shortly thereafter, the strobili elongated rapidly and they filled the inside space of the protective scales, thus loosing their pointed appearance. Male flowers collected on April 19, hat a rounded apex, and fully-formed microspores, with well developed air sacs, were present. The naked strobili were of a dull green color, and the inner bud scales were transparent. On April 26, the strobili hat ruptured the outside scales exposing the individual microsporangia, and the microspores had completed their second vegetative division. An additional ten days were needed for the microspores to complete their development, and dehiscing first took place on May 7. The ripe strobili had a total length of about 15 mm., had a reddish-brown appearance, and they had distinct scale-like terminal appendages on the sporophylls.

Part II. Cytology of Microsporogenesis.

Smears of anthers from flowers collected during December showed a mass of rounded cells within the microsporangium. These cells stained homogeneously without revealing any structural differentiation and no nucleoli were visible *(Fig. 6, A)*. This cell mass, generally termed sporogenous tissue, presumably originated through division and differentiation of a cell or cells within the sporangial primordium in the abaxial part of the microsporophylls. These cells formed a firm mass, and the individual cells of the

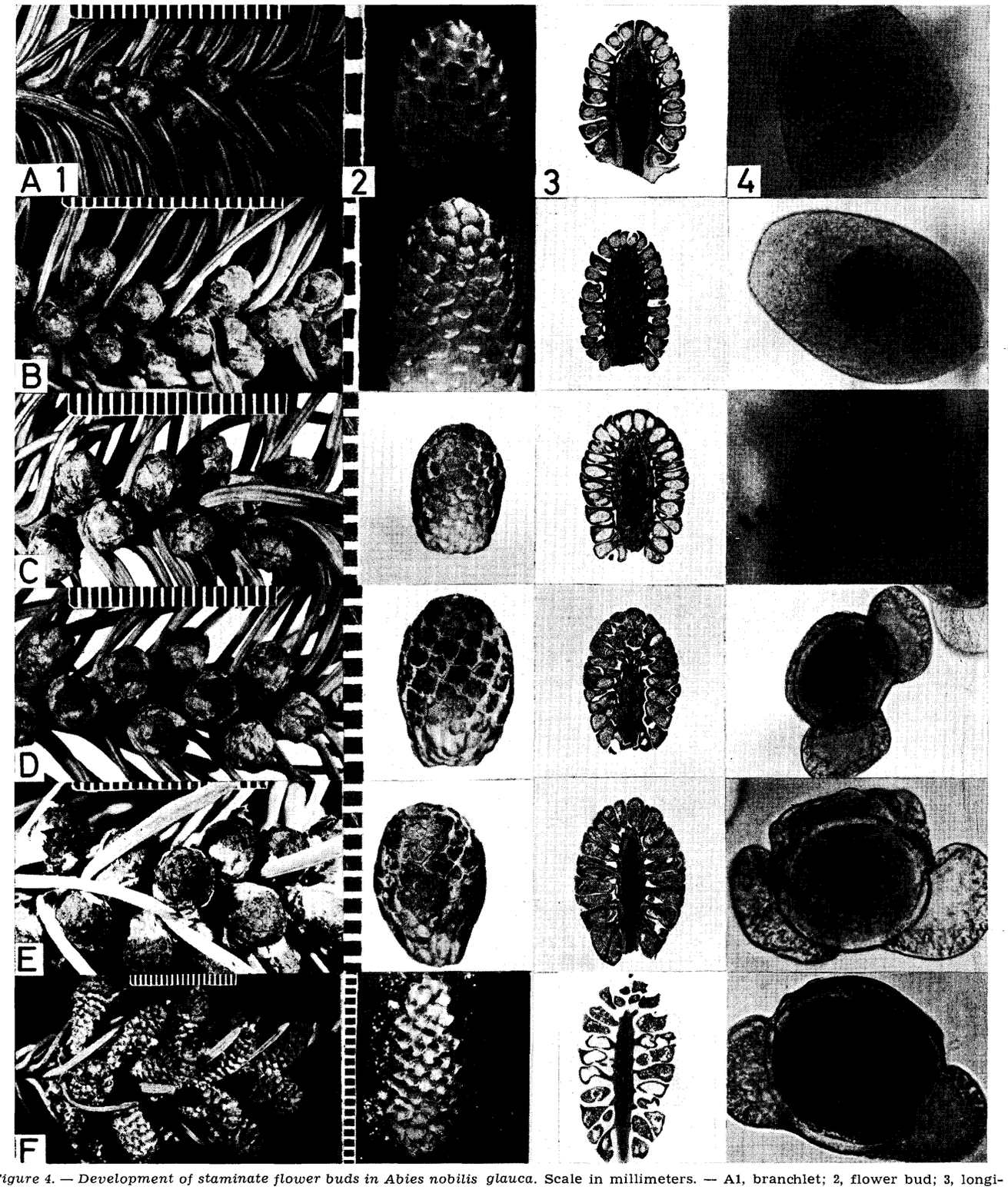

Figure 4. — Development of staminate flower buds in Abies nobilis glauca. Scale in millimeters. — A1, branchlet; 2, flower bud; 3, longitudinal section of bud; 4, stage of microsporogenesis. — *Dates of collection:* A, January 27; B, April 8; C, April 19; D, April 26; E, May 3; and F, May 18. — *Stages of microsporogensis:* A4, primitive archespore, 510×; B4, meiotic prophase of microspore mother cell, 540×; C4, anaphase of meiotic division, 470×; D4, first telophase of immature pollen grain, 310×; E4, third metaphase of immature pollen grain, 450×; F4, mature pollen grain at time of shedding, 340×.

sporogenous tissue could be isolated only with difficulty after hydrolysis in .1N HCl.

In the buds collected on January 25, the sporogenous tissue in all of the species had developed into primitive archesporial cells. These relatively large cells have an angular outline and are characterized by prominent nucleoli *(Fig. 6B)*. These nucleoli became visible during the development from sporogenous tissue to primitive archesporial cells and they only appeared when the primitive archesporial stage was reached. The number of nucleoli varied between three and eight, with the majority of cells containing three to five. One of these nucleoli was markedly larger

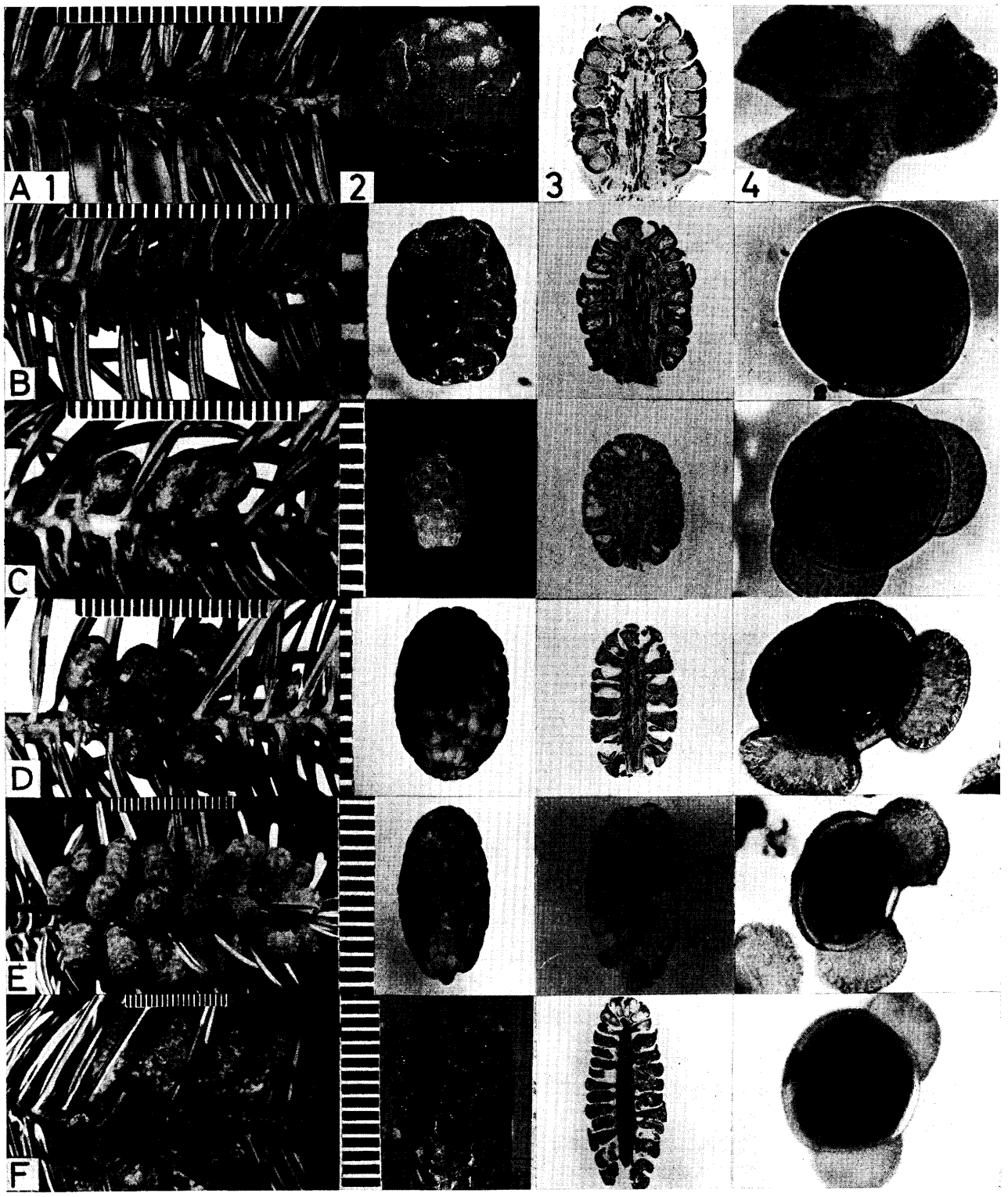

Figure 5. — Development of staminate flower buds in Abies×Boresii regis. Scale in millimeters. — A1, branch; 2, flower bud; 3, longitudinal section of bud; 4, stage of microsporogenesis. — *Dates of collection:* A, January 27; B, March 11; C, April 19; D, April 26; E, May 3; and F, May 7. — Stages of microsporogenesis: A4, primitive archespores, 390×; B4, meiotic prophase of microspore mother cell, 500×; C4, immature pollen grain, 520×; D4, third interphase of immature pollen grain, 360×; E4, third metaphase of immature pollen grain, 300×; F4, mature pollen grain at time of shedding, 240×.

in most of the cells. The duration of the archesporial stage varied considerably among the species, e. g. in *Abies sachalinesis* archesporial cells were observed only in buds collected on January 25, while in *Abies nobilis glauca* they were present over a period of two months, or until March 25. Only occasional divisions of an archesporial cell were observed.

The rounding of the primitive archesporial cell into a circular or oval cell marked the formation of the microspore mother cell *(Fig. 6C)*. These MMC contained a considerable number of starch grains, that tended to obscure the internal structure. With the exception of the starch grains, the MMC stained without structural differentiation for a brief period prior to the appearance of a reticulate

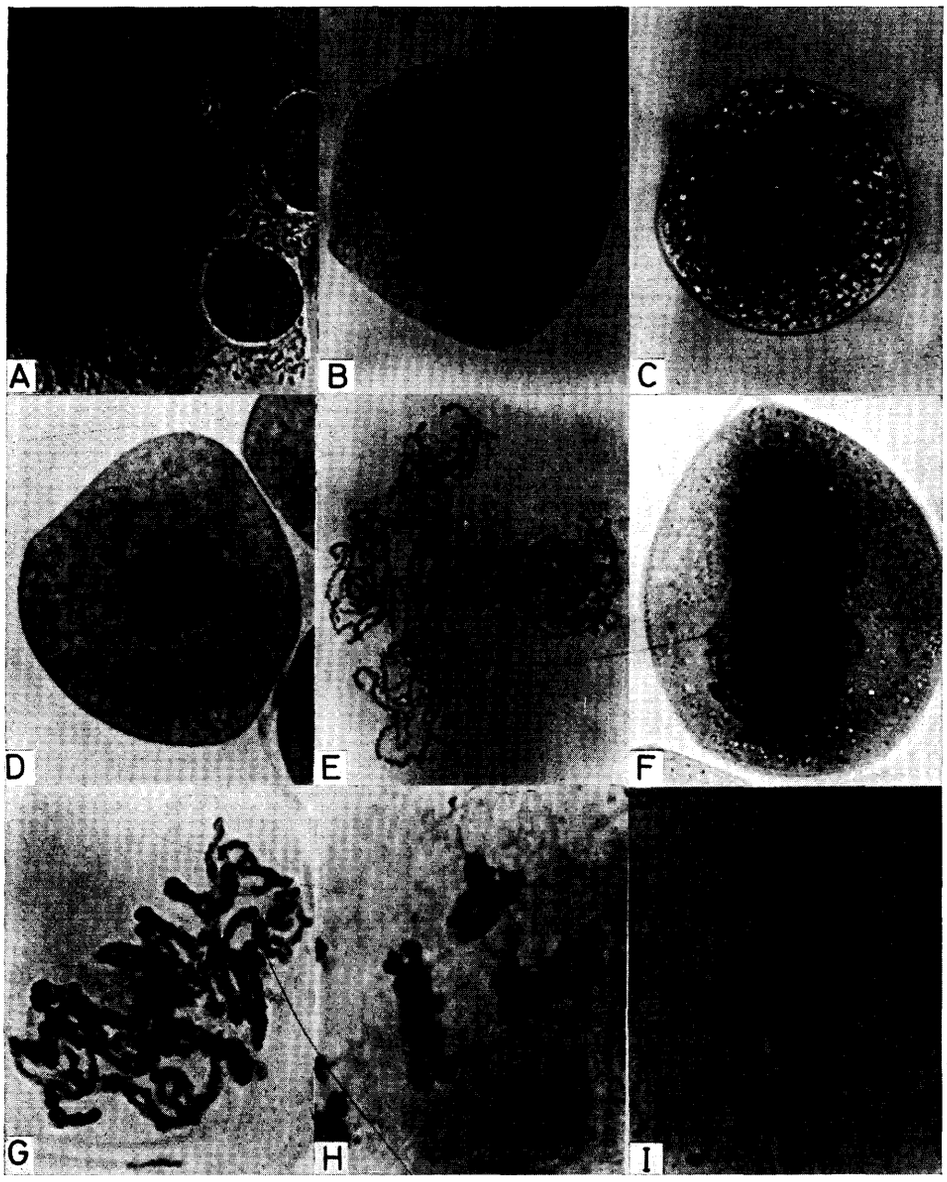

Figure 6. — Cytology of microsporogenesis: (N.G. = *A. nobilis glauca;* S = *A. sachalinensis;* H = *A. homolepis).* — A. Sporogenous tissue (N.G.), 230×; B. Primitive archespore (N.G.), 530×; C. Microspore mother cell (S), 550×; D—G. Meiotic prophases. D. (S), 870×; E. (S) 800×; F. (H). 500×; G. (H), 1180×; H. Diplonema (H), 790×; I. Diakinesis (H), 980×.

nucleus which marked the beginning of the meiotic prophase. It was not possible to achieve the microscopic resolution necessary to determine whether the first visible threads of chromatin were single (leptonema) or double strands (pachynema). In *Abies sachalinensis,* meiotic prophases were first visible in flowers collected on February 25, and similar stages were still present in collections made on March 24. The MMC of *Abies nobilis glauca* started their meiotic division only during the early part of April and continued over a period of about two weeks.

The initial stages of meiotic prophase showed chromosomal threads that were tangled and interwoven *(Fig. 6D).* The individual chromomeres along the full length of the chromosomes were clearly visible, as these bead-like structures stained much darker *(Fig. 6E).* Following the initial stages of meiotic prophase, the chromosomes contracted rapidly and their intertwining was clearly evident *(Fig. 6F)* The chromosomes in *Fig. 6G* have completed synapsis, and the paired threads indicate that meiosis has reached pachynema and the pairing of the homologous chromosomes has been completed. At this stage the individual chromosomes stained clearly.

After the pachynema stage was completed, microsporogenesis developed rapidly. Diplonema was characterized by very diffuse staining, despite the continued contraction of each chromosome pair. At this stage separation of the homologues took place, but the separation was not complete, as the chromosomes held together at one to several points. When held together at two points they gave the appearance of a single loop, and when held together at several points of contact they appeared as a series of loops *(Fig. 6H).* One large nucleolus was also present at this stage. Contractions and separation continued and the points of contact, or chiasmata, became pronounced. These loci are the potential points of crossing over or chromatid exchange. A representative plate of chromosomes with chiasmata is given in *Fig. 6I.* Alignment at the metaphase plate, anaphase separation, and formation of the dyad proceeded in rapid suc-

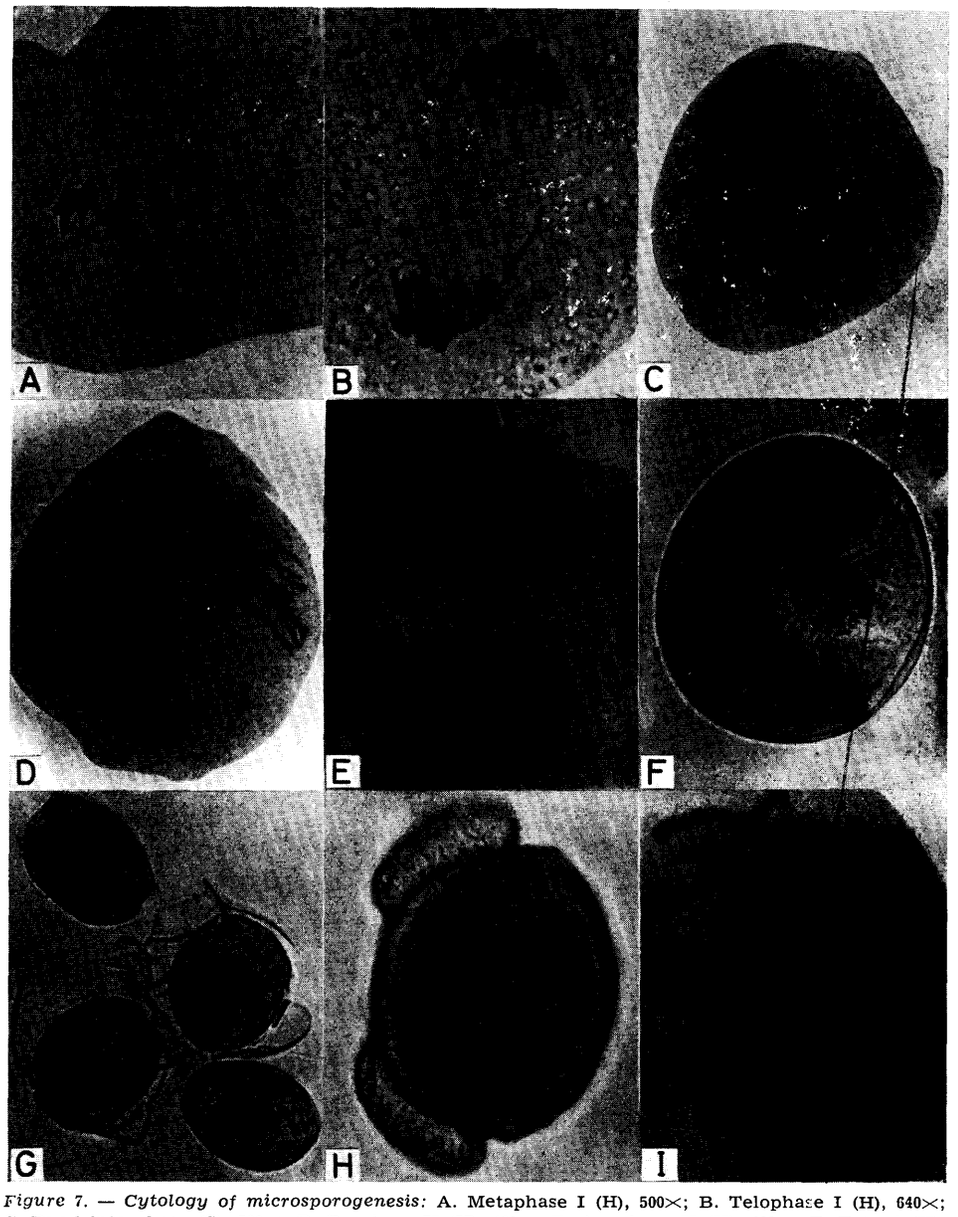

Figure 7. — Cytology of microsporogenesis: A. Metaphase I (H), 500×; B. Telophase I (H), 640×; C. Dyad interphase (S), 500×; D. Prophase II (H), 460×; E. Anaphase II (H), 400×; F. Tetrad (H), 440×; G. Microspores (S), 330×; H. Microspore prophase I (S), 780×; I. Chromosomes of I metaphase (S), 1160×.

cession *(Fig. 7A, B, C)*. Although mitotic division of the dyad nuclei was seldom synchronous *(Fig. 7 D + E)*, the four haploid (n) nuclei were separated from each other by the formation of cross-walls, and microspores of almost equal size developed *(Fig. 7, D, E, F)*. Rudimentary air sacs were already visible at the time when the pollen grains broke out of the enclosing MMC wall *(Fig. 7G)*. At this stage they contained a great number of starch grains.

In all the species under observation, meiosis proceeded rapidly and took less than one week for the development from pachynema to the immature microspore. During the week of meiotic division one could observe within one sporangium all of the developmental stages between pachynema and immature microspore. In *Abies sachalinensis* immature pollen grains were present in flowers collected during early April, while in *Abies nobilis glauca,* meiosis did not occur until the middle of April, and immature microspores were just breaking out of the MMC walls on April 25.

Rapid development of the pollen grains continued after they were freed from the MMC wall. The air sacs enlarged rapidly, and the nucleoli that were last visible during diakinesis reappeared during prophase of the first vegetative division *(Fig. 7H)*. The period between late prophase and early metaphase of the first mitotic division was very favorable to study the chromosomal morphology. In *Fig. 7I* are shown the chromosomes of *Abies sachalinensis* with three of the chromosomes having distinct satellites. These terminal bodies are attached to the main body by a thread of chromatin. Subsequent alignment on the metaphase plate, anaphase and telophase *(Fig. 8A)* followed the normal mitotic cycle. The dorsal one of the two telophase nuclei is forced against the dorsal intine, giving a flattened form that is characteristic of vegetative nuclei in the microspores of gymnosperms. The cytoplasm that surrounded the vegetative nucleus became indistinguishable as the vegetative cells were flattened *(Fig. 8B)*. The second mitotic cycle produced another vegetative cell which was flattened against the

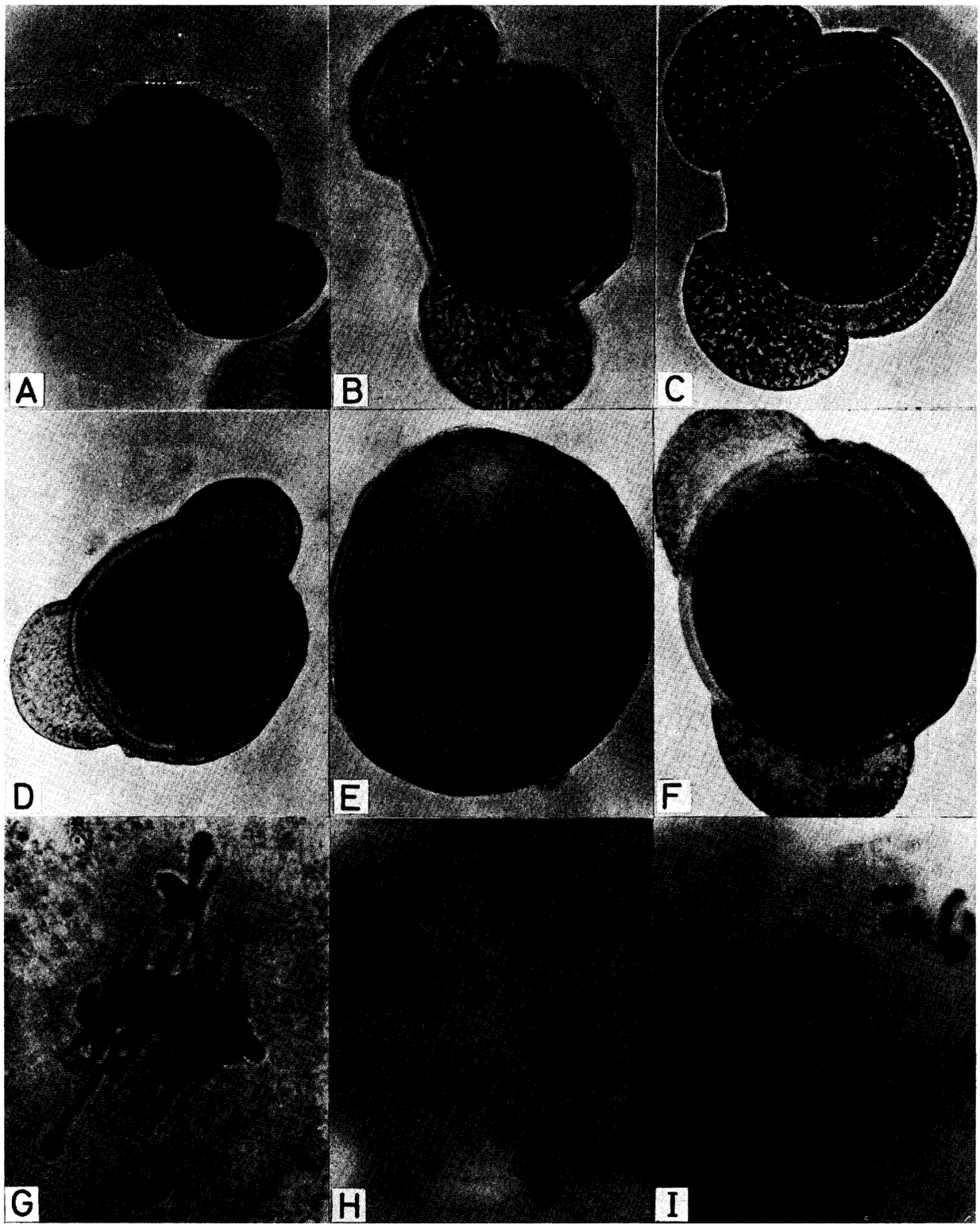

Figure 8. — Cytology of microsporogenesis: A. Microspore telophase I (N.G.), 580×; B. Microspore prophase II (N.G.), 700×; C. Microspore metaphase III (H), 500×; D. Microspore telophase III (S), 470×; E. Microspore interphase IV (S), 620×; F. Mature pollen (H), 500×; G. Abnormal metaphase I (N.G.), 1150×; H + I. Abnormal chromosome movement in anaphase I (N.G.), 610×; and 680×.

first vegetative cell *(Fig. 8C)*. A third mitotic cycle *(Fig. 8D + E)* produced a pollen tube nucleus and a generative nucleus. The latter divided into a stalk nucleus and body nucleus. At the time of pollen shedding, the microspores contained a total of five cells, with both the vegetative cells having been flattened against the dorsal intine *(Fig. 8F)*.

As mentioned previously, meiotic irregularities were observed in *Abies nobilis glauca*. These consisted of precocious movement of chromosomes during anaphase, formation of a bridge, and presence of acentric fragments. *Fig. 8G* is an example of precocious chromosome movement toward the poles during anaphase. Two pairs of chromosomes

have started their separation and movement, while the remaining chromosomes are still at the metaphase plate. In *Fig. 8H + I* are illustrations of a chromosomal bridge with the subsequent occurrence of an acentric chromatic fragment. This probably happened after a bridge was formed, and the strain during anaphase movement broke the bridge, leaving a portion of chromatid behind.

Discussion

During the 1959—1960 flower season there were large numbers of male flowers on the selected trees and no difficulty was encountered in completing all of the planned collections. On the other hand, although flower primordia were layed down during the 1960—1961 season, virtually no male flowers matured on the trees under study. This failure can probably be attributed to a severe winter when many of the flowers on ornamental trees were also killed by frost.

In the four species under observation, the microsporangiate strobili over-wintered in the primitive archesporial stage but the onset of further changes and differentiation proceeded at different rates in the various species. The initial stages of meiosis first took place in *Abies sachalinensis,* and pollen was shed after 9 weeks. For *Abies nobilis glauca* meiosis started only on April 5, but dehiscence took place on May 18. This difference in rates of development was well pronounced in the four species studied.

Various methods were tried to force the rate of microspore development by placing flower-bearing branches under various conditions of relative humidities and photoperiods in the greenhouse or in the laboratory. Flowers forced under these environments in either the greenhouse or laboratory enlarged considerably, and development was normal up to the second or third vegetative division. The rate of development was considerably speeded up by this artificial treatment, and on branches collected during the early part of February, microspores developed from archesporial cells in a period of 7 days. When flowers were forced during the beginning of March, the progress from MMC to pollen grains (3rd division) took only one week, but after the microspores had completed the third division, the strobili dried and turned brown, and no mature pollen was shed. The elongation and development of the flowers was faster when they were kept under high humidities in plastic bags, but this did not prevent the strobili from aborting. Similar results were obtained during attempts to force the ripening of catkins of longleaf pine (Mergen, 1954).

For the classification of plants it is generally recognized that the characteristics of the reproductive organs are stable and relatively uniform for a species, or variety. Of interest, however, was the fact that of the four *Abies sachalinensis* trees that were under study, the flowers were bright yellow on three trees at the time of pollen shedding, while on the fourth tree they had a reddish-brown color. The color within any one tree was uniform. Although flower color is variable within members of some plants, this is the first time that differences in the color of the male flowers within a species in the *Pinaceae* were observed by the authors.

For the species studied, over-wintering took place in the primitive archesporial stage and the gradual transition of these cells into microspore mother cells occurred between February and April. In his report on the development of pollen grains in *Abies balsamea,* Hofmeister (1848) reported that he already observed pollen mother cells in the microsporangia during the fall, and that they remained in this condition during the winter. However, according to his description and his illustration of the cell contents, these cells could probably be considered as being in the archesporial stage according to our current terminology. At this stage most of the details were masked by the presence of a large number of starch grains, which disappeared when the reticulate prophase nucleus of the meiotic division occurred. It was of considerable interest to be able to observe and photograph the chromomeres along the individual chromosomes. This stage was followed by a distinct contraction and intertwining of the chromosomes. Considerable difficulties were encountered in staining cells in the diplotene stage, and only diffuse staining was achieved.

In this study, pollen shedding took place after the generative nucleus had divided into a stalk nucleus and body nucleus, and in no instance was a division of the body nucleus into sperm nuclei observed. This is contrary to the findings by Hutchinson (1914), who observed the division into male nuclei in about 10 percent of the gametophytes of *Abies balsamea.*

In their description of the chromosomes in two species of *Abies* Sax and Sax (1933) did not report on the presence of satellites. Actually the presence of satellites in the *Coniferales* has not been observed previously. In *Abies sachalinensis* satellites were clearly visible on three chromosomes. Further work is being done to see whether or not SAT-chromosomes are present in other species of *Abies,* and controlled crosses have been made to obtain some information on the stability and inheritance patterns of these distinct chromosomes.

The irregularities that were observed during meiosis in *Abies nobilis glauca* might help to explain the subsequent behavior of the pollen that was obtained. When pollen was used in an intraspecific cross, and in a selfing, no viable seed was obtained. Twenty-nine seeds that were obtained from these crosses were examined, 20 percent were empty and 80 percent contained embryos that had dried and were in a shriveled condition. However, when *Abies nobilis glauca* was used as a female parent, viable seed was obtained. Further work is being planned with these trees, as this might be one of the instances where the incompatibility of a species as a male parent can be explained on the basis of gross physical abnormalities during chromosome movements, preceding pollen shedding.

Summary

Two aspects of staminate flower phenology and pollen formation in *Abies sachalinensis, A. homolepis, A. nobilis glauca* and *A. × Boresii regis* are described. In *Part I,* the external and internal changes that take place during the formation of pollen grains are illustrated with photographs for six collection dates. In *Part II,* the cytology of microsporogenesis is described in detail and illustrated with the aid of photomicrographs. Meiotic irregularities were observed in *A. nobilis glauca.* The precocious movement during anaphase resulted in the formation of bridges and acentric fragments.

Three chromosomes of *A. sachalinensis* had distinct satellites, and they are illustrated in a metaphase plate.

Zusammenfassung

Titel der Arbeit: *Mikrosporogenesis bei Abies.*

Phänologie und Pollenbildung werden bei männlichen Blüten von *Abies sachalinensis, A. homolepis, A. nobilis glauca* und *A. × Boresii regis* beschrieben. Im *Teil I* wer-

den äußere und innere Veränderungen, die sich während der Ausbildung der Pollenkörner ergeben, mit Photographien von sechs Probenahme-Daten belegt. Im *Teil II* ist die Zytologie der Mikrosporogenesis im einzelnen beschrieben und durch Mikrophotographien veranschaulicht worden. Bei *A. nobilis glauca* wurden meiotische Unregelmäßigkeiten beobachtet. Vorzeitige Chromosomenbewegung während der Anaphase ergab Brückenbildungen und einseitige Fragmente.

Drei Chromosomen von *A. sachalinensis* hatten klar sichtbare Satelliten. Sie wurden in einer Metaphasenplatte abgebildet.

Résumé

Titre de l'article: *Formation des microspores chez les Abies.*

L'article décrit deux aspects de la phénologie des fleurs mâles et de la formation du pollen chez *Abies sachalinensis, A. homolepis, A. nobilis glauca* et *A. boresii regis.* Dans la première partie, les changements externes et internes qui interviennent au cours de la formation des grains de pollen sont illustrés par des photographies prises à six dates différentes. Dans la deuxième partie, la cytologie de la formation des microspores est décrite en détail et illustrée par des microphotographies. On a observé des irrégularités à la meiose chez *A. nobilis glauca.* Le mouvement précoce au cours de l'anaphase aboutit à la formation de ponts et de fragments acentriques.

Trois chromosomes d'*A. sachalinensis* ont des satellites distincts, comme on peut le voir dans une photographie prise à la métaphase.

Literature Cited

Hofmeister, W.: Über die Entwicklung des Pollens. Bot. Zeitung 6 (23): 425—434, 649—658, 670—674 (1848). — Hutchinson, A. H.: The male gametophyte of *Abies*. Bot. Gaz. 57: 148—153 (1914). — Hutchinson, A. H.: Fertilization in *Abies balsamea*. Bot. Gaz. 60: 457—472 (1915). — Mergen, F.: Improving the early growth of longleaf pine. Naval Stores Rev. 64 (3): 12, 13, 21 (1954). — Mergen, F., and Lester, D. T.: Colchicine-induced polyploidy in *Abies*. For. Science *(In print)* (1961). — Miyake, K.: Contribution to the fertilization and embryogeny of *Abies balsamea*. Beihefte Bot. Centralblatt 14: 134—144 (1903). — Sax, K., and Sax, H. J.: Chromosome number and morphology in the Conifers. Jour. Arnold Arboretum 14: 356—375 (1933).

Referate

Petrini, S.: **De två äldsta svenska tallproveniensförsöken.** (Die zwei ältesten Kiefern-Provenienzversuche in Schweden.) Medd. fr. Stat. Skogsforskn. Inst. **48**, (11) 1—49 (1959).

Zwei von Schotte im Jahre 1904 angelegte *P. silvestris*-Provenienzversuche wurden im Herbst 1957 gemessen und ausgewertet. Eine der Versuchsflächen liegt in Ollestad, Västergötland (57° 58'), die andere in Torared, Halland (56° 41'). Die Flächen enthalten jeweils 35 bzw. 9 schwedische Herkünfte von 56° 40' (Småland) bis 63° 5' (Jämtland). Außerdem wurden in Torared die deutschen Provenienzen Darmstadt (Hessen) und Eberswalde (Mark Brandenburg) angebaut. — Die Ergebnisse bestätigen im allgemeinen die aus anderen Kiefern-Provenienzversuchen bekannt gewordene Tendenz. Nördliche Herkünfte haben weniger Stammkrümmungen und feinere Beastung. Hingegen ist die Astreinigung bei nördlichen Kiefern ungünstig. Hier reagieren südschwedische und märkische Kiefern günstiger. Hinsichtlich der Massenleistung liegen die südliche Provenienz Sunnerbo (Småland) und die mittelschwedische Provenienz Tiveden (Västergötland) mit 11,0 fm lfd. jährl. Zuwachs pro ha an der Spitze und die Norrlandsherkünfte mit 3,9 bzw. 5,8 fm/ha/Jahr am Ende. Wegen der höheren Qualität ist die Kiefer aus Tiveden der aus Sunnerbo vorzuziehen. Die Darmstädter Kiefer fällt durch den größten Brusthöhendurchmesser auf. Schütt

Pitcher, J. A.: **Heteroplastic grafting in the genera Acer, Fraxinus, Picea and Abies.** Proc. 7th Northeastern For. Tree Impr. Conf., Burlington, Vt. 1959, 52—57 (1960).

Die Unterlagen waren 5 j. v., die Reiser gehörten einem einzigen Klon der betreffenden Art an. Bei den Nadelbäumen wurde jeweils ein Reis unter die Rinde gepfropft. Bei den Laubbäumen verwandte man 2 Reiser und pfropfte eines unter die Rinde, für das andere wandte man Flaschenpfropfung an. Anschließend wurden alle Pfropflinge mit Polyaethylen und Papier eingetütet. — Bei *Abies* (Unterlage *A. balsamea*) wuchsen die Reiser von 9 Arten im Gewächshaus zu 85% an (Freiland 80%). Die Ergebnisse bei den anderen Gattungen waren: *Picea* (Unterl. *P. abies*, 11 Arten) Anwachsprozent 63 bzw. 25, *Fraxinus* (Unterl. *F. americana*, 5 Arten) Anwachsproz. 17 bzw. 64, *Acer* (Unterl. *A. saccharum*, 17 Arten) Anwachsproz. 5 bzw. 6. — Die Anzahl der Pfropfungen betrug im Durchschnitt aller Arten unter 10 Stück.

Entspitzen der Tannenknospen oder ihre Behandung mit alkoholischen Lösungen wirkte sich nicht förderlich aus. Bei *Picea* öffneten sich die Knospen der Reiser erst nach 75 bis 100 Tagen. *P. polita* machte in der laufenden Vegetationsperiode überhaupt keinen neuen Trieb, doch zeigten sich Reis und Unterlage gut verwachsen. Hattemer

Privalov, G. F.: **Lebensfähigkeit von Birkensamen im Verlauf der Reife und der Lagerung.** Botan. Zurnal **45**, 149—151 (1960). *[Russisch.]*

Berichtet wird über Untersuchungen an *Betula verrucosa, B. pubescens* und *B. pubescens* f. *rhombifolia* im Alter von 15 Jahren: Die Bestimmung der Lebensfähigkeit der Samen wird durch Keimung voller Samen auf Filterpapier festgestellt. Sie erfolgte sofort, nach 1 Jahr und nach 3 Jahren. Volle Samen keimen zu 94 bis 99%. Das Trocknen der Samen erhöht ihre Lagerfähigkeit. Die Herabsetzung der Feuchtigkeit auf 7,4% des Lufttrockengewichtes verringert nicht die Keimfähigkeit. Einen Monat vor der Vollreife, also bereits im Juli, haben die Samen ihre volle Triebkraft erreicht. Bei Lagerung unter trockenen Bedingungen bleibt die Keimfähigkeit 3 und mehr Jahre erhalten. In den ersten Lebensjahren übertrifft *Betula verrucosa* die *B. pubescens* im Höhenwachstum, während umgekehrt die *B. pubescens* einen größeren Dickenzuwachs aufweist. v. Dellingshausen

Radler, F.: **Erfahrungen mit dem Anbau von Sequoia gigantea.** Holz-Zentralblatt **86**, 677—678 (1960).

Als Fortsetzung der Betrachtungen früherer Berichte über den Anbau dieser Baumart in Deutschland ergänzt hier Verf. die Hinweise auf positiv zu bewertende Anbauerfolge. Einige dieser Kulturen überlebten zum Teil außerordentlich ungünstige klimatische Bedingungen. Seitz

Rahte: **Die herkunftsmäßige Differenzierung des Angebotes von forstlichem Saat- und Pflanzgut.** Allg. Forstztschr. **15**, 20—21 (1960).

Verfasser, der Mitglied des nach Inkrafttreten des forstlichen Saat- und Pflanzgutgesetzes gebildeten Kontrollausschusses ist, erörtert die Unterschiede, die sich für die Praxis gegenüber den aus dem alten Gesetz resultierenden Regelungen ergeben. Insbesondere kann anerkanntes Saatgut nur für die vom Gesetz erfaßten 16 Baumarten geliefert werden und für diese ausschließlich anerkanntes Material. Für alle anderen Baumarten kann keine Anerkennung ausgesprochen werden. Weiter wird in Zukunft nur noch das Herkunftsgebiet angegeben, nicht aber der anerkannte Waldteil, aus dem es stammt. Neben dieser gesetzlich vorgeschriebenen Kennzeichnung kann die „Deutsche Kontrollvereinigung für forstliches Saat- und Pflanzgut" Sonderbezeichnungen (Angabe des Herkunftsorts) anfügen sowie Herkunftsbezeichnungen auch für nicht anerkennungsfähiges Saatgut festlegen. Stern