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CONTENTS

	<i>Page</i>
RESEARCH SUMMARY	ii
INTRODUCTION	1
NATURAL DISTRIBUTION	2
TAXONOMY	3
SEXUAL REPRODUCTION	3
Reproductive Development	3
Anomalous Flowering	6
Sex Ratio	6
Cut-Branch Technique	6
Seedling Production	7
ASEXUAL REPRODUCTION	7
Natural Suckering	7
Rooting	8
Air Layering	8
Stem Cuttings	8
Root Sprouts and Sucker Cuttings	8
Grafting	9
Tissue Culture	9
GENETICS AND BREEDING	11
Natural Variation and Inheritance	11
Growth, Form, and Wood Properties	11
Specific Gravity	12
Fiber Length	12
Wood and Pulp Properties	13
CYTOGENETICS	13
Chromosome Numbers	13
Triploidy	14
Tetraploidy	14
Monoploidy	15
INTERSPECIFIC HYBRIDS	16
Natural Hybridization	16
Artificial Hybridization	16
TREE IMPROVEMENT PROGRAMS	17
Determining Tree Improvement Goals	17
Selection of Parent Trees	18
Parent Tree and Progeny Evaluation	18
Quaking Aspen for the Year 2000	19
LITERATURE CITED	20

RESEARCH SUMMARY

Quaking aspen (*Populus tremuloides* Michx.) is the most widely distributed tree in North America and exhibits considerable variation in form, growth rate, and wood properties. Ecologically, the species occupies an important place in forest succession and is a species that can be maintained on an area through the use of simple inexpensive forest management techniques.

Quaking aspen is characteristically dioecious and crosses readily with species of *Populus* within the section *Leuce*. Easily propagated and easily hybridized, the species has been extensively investigated. Wide geographic distribution has resulted in the description of a number of varieties. Quaking aspen tetraploids, triploids, and monoploids have been reported. Naturally occurring and artificially

produced triploid quaking aspen exhibit good form, rapid growth, and improved fiber properties. Research on natural variation and heritability suggests considerable genetic gain will result from tree improvement programs emphasizing height and diameter growth and such wood properties as specific gravity and fiber length.

Quaking aspen, particularly in the Lake States Region, is providing the area with a valuable resource. Properly oriented genetics and intensive management research programs stressing rapid growth, "juvenile" wood quality, response to intensive management, and improved utilization can be expected to make aspen an even more valuable resource in the future.

Height growth

GENETICS OF QUAKING ASPEN

Dean W. Einspahr and Lawson L. Winton ¹

INTRODUCTION

Since quaking aspen (*Populus tremuloides* Michx.) is an aggressive pioneer species, many of the present stands have originated as a result of natural or man-made catastrophes. Ecologically, the species occupies an important place in forest succession, readily colonizing burns, cut-over sites, and old field areas and, like a number of hardwood species, tends with time to improve the quality of the sites it occupies. Although quaking aspen is classified in many circles as a "weed species" of little commercial value, use of aspen pulpwood in the Lake States Region increased from 4 percent of the total pulpwood harvest in 1930 to 50 percent or a harvest

of 4.1 million cubic meters in 1969 (Keays 1972). Predicted requirements for hardwood pulp indicate major increases can be expected by the year 2000. One of the most promising possibilities for economically meeting part of the predicted hardwood requirement is through the use of genetically improved trees and such intensive forestry practices as fertilization, irrigation, and chipping in the woods operations. Quaking aspen appears to be well suited for such an extensive forestry system (Einspahr 1972) and increased use of such approaches can be expected.

NATURAL DISTRIBUTION

Quaking aspen, the most widely distributed tree in North America, grows from Newfoundland and Labrador west across northern Canada to northwestern Alaska (fig. 1). In the East and Midwest, scattered locations of quaking aspen have been reported as far south as western Virginia and northern Missouri. In the Rocky Mountains of western North America, quaking aspen grows from northern Mexico north to Alaska. Extensive stands are found growing at elevations of 2000 to 3000 meters in Colorado, the Great Basin, Arizona, New Mexico, in some areas in the northern Rocky Mountains, and in Canada.

Quaking aspen, although sensitive to variations in site, grows on a wide variety of upland soils ranging from stoney sands to heavy clays.² Low moisture and restricted nutrient levels result in reduced growth on the droughty soils. Some of the best quaking aspen stands occur on medium-textured, gray, glacial drift soils of southern Manitoba and Sas-

katchewan and northeastern Minnesota (Stoekeler 1948). Figure 2 illustrates such a stand growing in northeastern Minnesota. In the Lake States Region and Eastern United States, quaking aspen is found growing with a wide variety of tree species. On the drier upland soils associated species include Norway pine, jack pine, paper birch, and bigtooth aspen. On the medium-textured, moist soils associated species include the northern hardwoods (basswood, oak, maple, etc.), balsam poplar, white pine, white spruce, and balsam fir. In the Western United States and Canada a similar pattern exists with quaking aspen being associated with paper birch, lodgepole pine, and ponderosa pine on the drier sites; and with balsam, poplar, black cottonwood, alder, white fir, and white, sitka, Engelmann, and blue spruce on the moister situations.

Quaking aspen on upland soils of the Lake States Region are found over extensive areas and are associated with bigtooth aspen and paper birch. The magnitude of the importance of aspen in the Lake States and Northeastern United States is illustrated by the fact that the aspen-birch type is estimated to occupy 6.3 million hectares (15.5 million acres) in the Lake States Region (Chase, *et al.* 1970; Stone and Thorne 1961; and Stone 1966) and the total acreage in the aspen-birch type in the northern forests of the United States is reported to be approximately 9.6 million hectares (23.7 million acres) (Hair and Spada 1970).

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²For a more complete review of the silvics of quaking aspen, the reader is referred to the publication by Strothmann and Zasada (1957) "Silvical Characteristics of Quaking Aspen," "Silvics of Forest Trees of the United States," U.S. Dep. Agric. Handb. 271 (1965) compiled by H. A. Fowells, and the several articles dealing with the silvics and ecology of aspen in the "Proceedings of the Aspen Symposium," U.S. Dep. Agric. For. Serv. (1972).

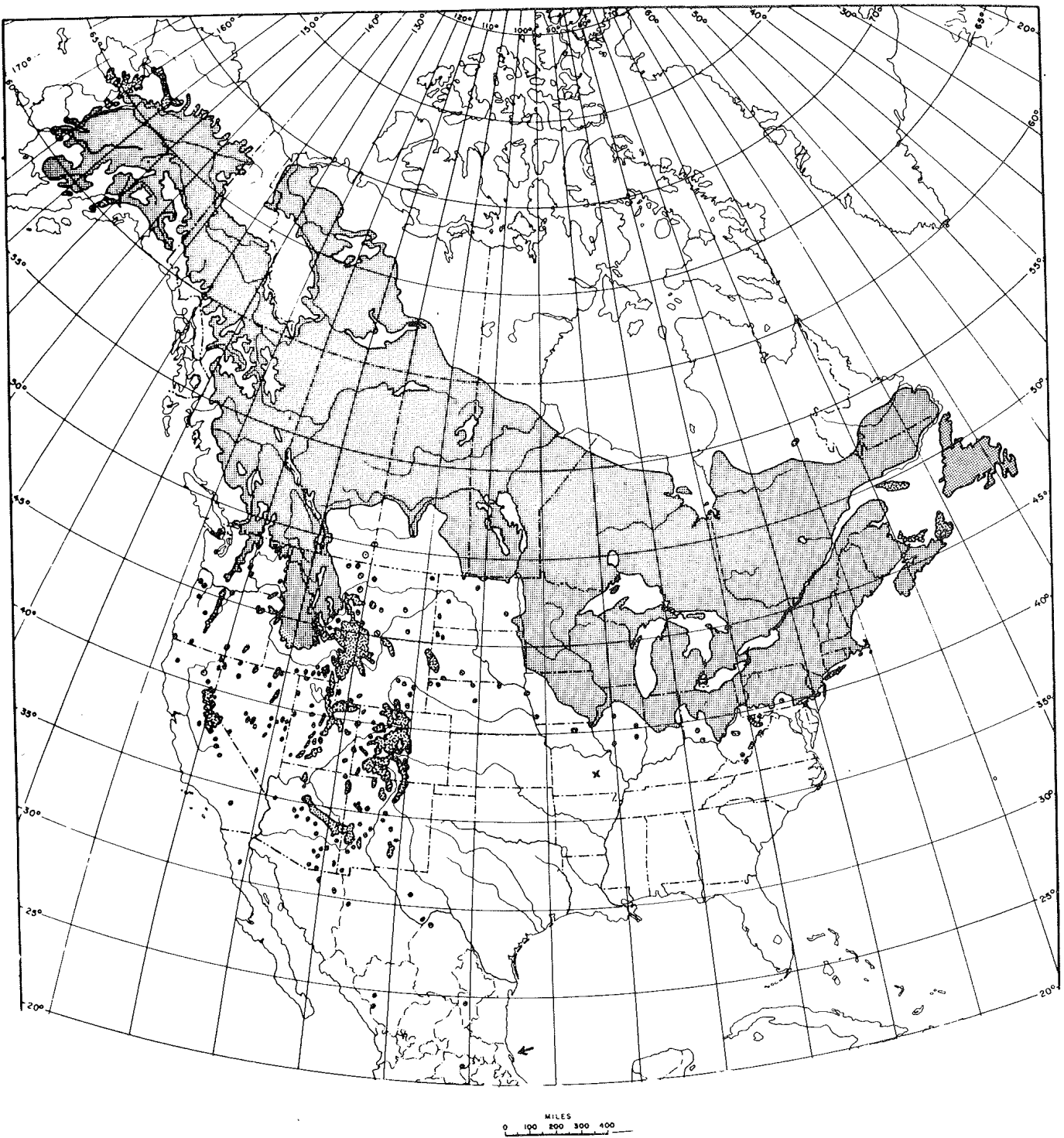


Figure 1.—Botanical range of quaking aspen.

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Figure 2.—Mature quaking aspen, northeastern Minnesota.

TAXONOMY

Several varieties of quaking aspen have been described, but because of the large interclonal variation found throughout the range of the species, a number of the intraspecific scientific names that have been created have not been accepted (Barnes 1969). The aspen of the mountains of Colorado (*P. tremuloides* var. *aurea*, Tid.) is the most widely accepted variety. This variety is distinguished from the typical by shorter calyces, longer anthers, and a deep golden fall coloration (Sudworth 1934; Brayshaw 1965). Other taxa that have been described in some detail include an eastern forma *pendulata*, Jaeger and Beissner (Fernald 1950); an eastern forma *reniformis* (Tid.) (Fernald 1950) and a coastal variety *vancouveriana* (Trel.) Sarg. which is restricted to Vancouver Island, B.C. (Sudworth 1934).

Taxonomists have divided the genus *Populus* into five sections. Quaking aspen, along with the closely related species of bigtooth aspen (*P. grandidentata* Michx.) and European aspen (*P. tremula* L.), have been placed in the subsection *Trepidae* of the section *Leuce*. Also included in this subsection are the Asiatic species *P. sieboldii* Miq. and *P. davidiana* Dode. Other *Populus* species classified within the section *Leuce*, but in the subsection *Albidae*, include *P. alba* L. and *P. canescens* Sm. (FAO 1958).

SEXUAL REPRODUCTION

Reproductive Development

Quaking aspen is generally dioecious, with male (staminate) and female (pistillate) flowers borne on separate trees, arranged along modified spikes called catkins or aments. Both types of flowers appear several weeks before the leaves, and female trees generally flower before male trees (Maini 1972). In male flowers, the stamens vary in number from 4 to 10 per scale in the Chicago area (Nagaraj 1952) to 6 to 12 in southern Ontario, Canada (Maini and Cayford 1968), and are partially enclosed by an obliquely-lengthened cup-shaped disk attached to the rachis by a short pedicel.

On the female catkin, single ovaries per flower are glabrous, conical and bicarpellate, with the lower two-thirds enclosed by a collar disk. A short style supports two stigmas, each branching into slender lobes. Each ovule develops into one capsule which is one-celled, narrowly conical, and has a green warty appearance. The capsules elongate to 3 to 5 mm in length after pollination and become slightly curved. The number of capsules per catkin ranges from 90 to 100 in the Central United States with 6 to 7 seeds

each, to 70 to 80 in Canada with about 10 seeds each.

The dates of floral initiation vary throughout the transcontinental range of quaking aspen, but the entire floral cycle is completed in 1 year. In Connecticut, both male and female primordia were initiated in mid-June, shortly after the first flush of vegetative growth (Lester 1963a). Pistils and stamens were formed by July, causing the floral buds to swell larger than the vegetative buds, with the male buds much plumper than the female buds. Flowers overwintered in either the microspore-mother-cell (male) or the archesporium (female) stage.

In southeastern Michigan, microsporogenesis generally occurs in March (B.V. Barnes, personal communication) and it occurs in central Wisconsin in early April. An unreported correlation has been observed for the past several years, of the degree of warming with microsporogenesis and anthesis (Winton, unpublished data). As the spring weather warmed in central Wisconsin, early prophase stages developed slowly in late March and early April. This was followed by the rapid completion of meiosis to the tetrad stage, usually on the first day when the

air temperature in the shade reached 10° C. At least for the few trees sampled, tetrad production occurred in both large and small male buds on lower branches at 10° C, but not at 9° C. The weather generally cooled for the next 2 weeks, and pollen was shed (anthesis) when the air temperature again reached 10° C. In southern Canada, flowering occurs in early April (Maini 1972), and, further north at Edmonton, Moss (1960) found that the average date of flowering for quaking aspen has been April 25 for the period 1928–1960, and occurred only after 6 days of air temperatures of 12° C or more. An increasing heat requirement for flowering in more severe climates could be an important survival trait, but provenance studies are needed to determine if such is the case.

Megagametogenesis occurs in female trees immediately after the microspores form in the male trees and while they are still in the tetrad stage (Nagaraj 1952). The megagametophyte is of the 8-nucleate Normal Type, which includes a haploid egg nucleus and a fused diploid polar body. After pollination, the pollen tube grows quickly down inside the style and through the micropyle. This action is followed by double fertilization of the egg nucleus and the polar body, each by one haploid male nucleus. The triploid polar nucleus then develops into the nutritive endosperm, causing rapid swelling of the ovule. The epidermal cells of the placental stalk divide longitudinally and elongate into single hair cells, causing rapid swelling of the capsule. The fertilized egg (zygote) then divides and develops into the seed embryo of the Onagrad Type.

Flower forcing is essential to meaningful crossing programs, and knowledge of the pattern of development is often necessary in order to give specific treatments during or after pollination. To study forcing development, female flowers were brought into the greenhouse, pollinated, and forced in a growth chamber (Winton 1968a). Sectioned capsules showed that pollen germinated on the stigmas 6 hours after pollination, when the receptive megagametophyte contained an egg nucleus and the polar body was in either the unfused (fig. 3, *A*) or the fused (fig. 3, *B* and 3, *C*) stage. Fertilization occurred after 30 to 48 hours (fig. 3, *D*), followed by the initial development of the endosperm (fig. 3, *E*). The first mitotic division of the zygote (fig. 3, *F*) occurred 54 to 72 hours after pollination. Seeds were shed 21 days after pollination when forced in the growth chamber, but in the field seed is shed in May, about 3 to 4 weeks after anthesis (Einspahr and Joranson 1960).

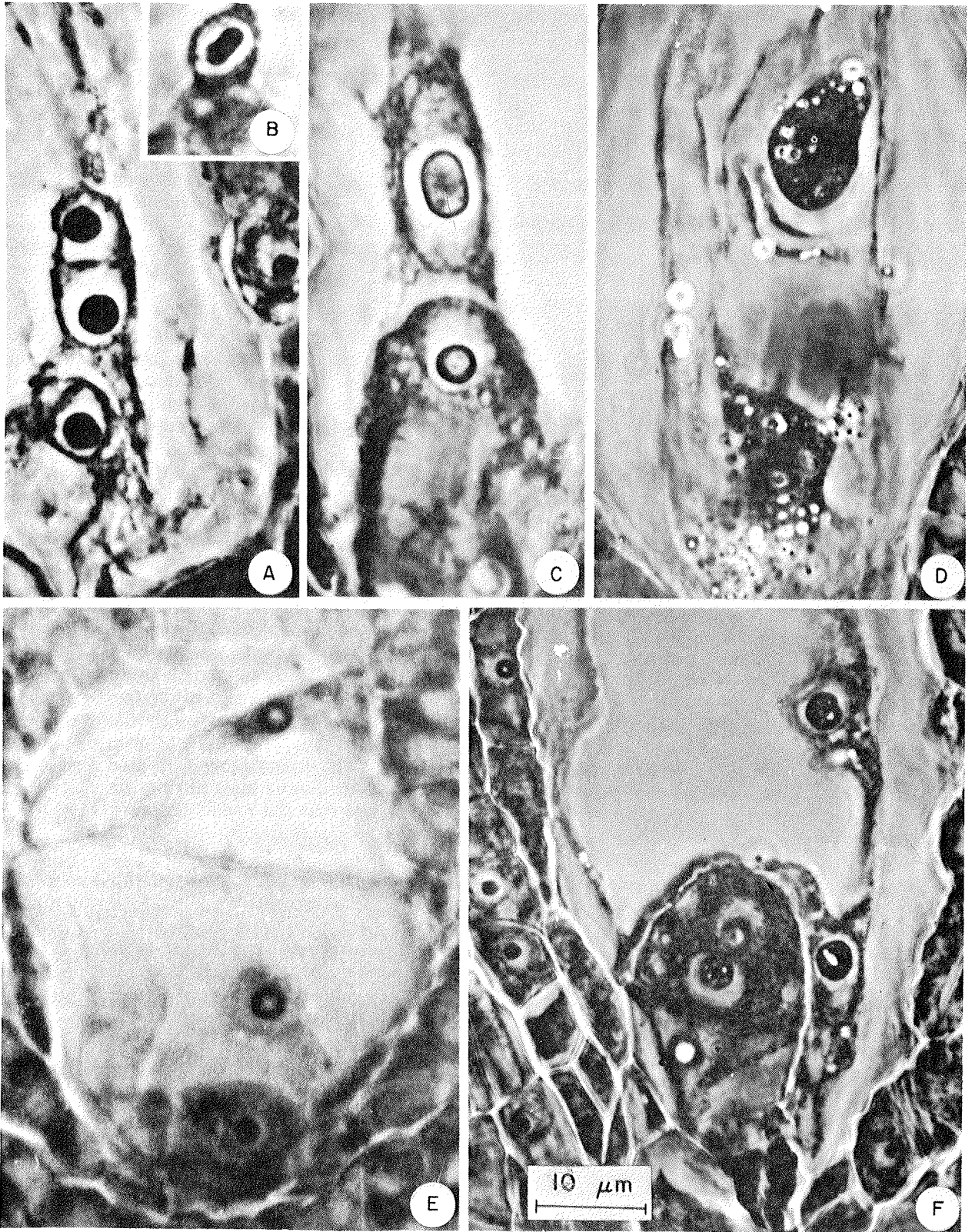
Seeds are pear shaped, about 1-mm long (USDA Forest Service 1948), and are distributed by wind with the aid of the long hairs of the placenta which remains attached to each seed. Hair is easily re-

moved by tumbling (Harder 1970), but flash-burning is not recommended because of the unknown extent of seed injury. Most quaking aspen seed are retained on a 40-mesh screen with 0.5-mm pores, and 1,000 40-mesh seeds weigh about 0.13 gram (Harder 1970, Maini and Coupland 1964). In natural seed stands, good seed crops are typically attained after 20 years and maximum yields occur between 50 and 70 years (USDA Forest Service 1948). Some clones flower every year, especially open-grown male trees (B.V. Barnes, personal communication), and the difference in times of flowering appears to be a fairly good trait that can be used to delineate adjacent clones (Maini 1972).

Flowering generally starts earlier in plantations, at 10 to 15 years of age in Canada (Maini and Coupland 1964) and at 8 to 10 years of age in Wisconsin. A good seed crop can be expected about every 2 years, and apparently a hot dry summer stimulates a large seed crop the following year, as has been suggested for many other forest trees (Busgen and Munch 1929; Fraser 1958). Langhammer (1963) concluded that relatively more heat is necessary for the initiation of female bud primordia than for male primordia, perhaps explaining why male trees seem to flower more frequently than do female trees. However, more temperature-related studies are needed for all phases of flowering.

Germination rates of 95 percent or more can be maintained in freshly collected seed for only 2 to 3 weeks at room temperature. However, high germination can be retained for up to 1 year by refrigerating seed in sealed containers (Kittredge and Gevorkiantz 1929) or by storing over calcium chloride at either 4.5° C (Benson and Harder 1972) or at -5° C (Moss 1938). Benson and Harder (1972) also removed seed from -11° C storage at yearly intervals during a 4-year period, and found that the germination dropped slowly from the original 99 percent to values ranging from 75 percent to 94 percent. On the other hand, the germination of control seed stored in the refrigerator dropped steadily over the same period to 11 to 51 percent after 4 years.

Seeds are not dormant, but will germinate within 1 to 2 days after shedding at temperatures between 0° C and 35° C (Faust 1936). During the first few days after germination the seedlings absorb most of their water through hypocotyl hairs (Moss 1938). Thus, water is a limiting factor for seedling establishment, a rare event in nature considering the millions of seeds produced each year. However, seedling origin has been established for both western quaking aspen (Ellison 1943; Larson 1944; Barnes 1967) and for eastern quaking aspen (Pauley and Mennel 1957; Andrejak and Barnes 1969). Seedling reproduction may be the exception at the present time, but the seed origin of most quaking aspen



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Figure 3.—A study of forcing development: A, Quaking aspen female gametophyte, two polar nuclei above the egg cell; B, fusion of the polar nuclei (cross section); C, polar body above egg cell; D, fertilization of polar body (top) and egg cell (out of focus below); E, first division of the polar body, zygote in micropylar cavity; and F, first transverse mitotic division of zygote after development of the endosperm from the polar body (Winton 1968a).

clonal stands today may date back to the close of the last century, when devastating fires and other catastrophes swept many areas of the Northeastern United States (Schreiner, personal communication) as well as in the Lake States Region. Natural, direct seeding occurred from quaking aspen survivors after the fires, followed by vegetative sucker propagation.

Anomalous Flowering

Selfing is impossible for completely dioecious species. But, fortunately, the selfing of individual aspen trees may someday become a useful breeding tool because of the discovery of occasional trees having both sexes. These deviations from dioeciousness include, (1) trees with both male and female unisexual catkins, (2) trees with catkins having both male and female unisexual flowers, (3) trees having perfect flowers containing both male and female parts, and (4) trees with various combinations of the above. The first bisexual quaking aspen tree having perfect flowers (fig. 4) was reported by Erlanson and Her-



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Figure 4.—A perfect flower from a hermaphroditic quaking aspen in which both the female (left foreground) and male (right background) floral parts occurred in the same flower.

mann (1927) in Michigan. Subsequent combinations of hermaphroditism were reported by Santamour (1956), Pauley and Mennel (1957), Einspahr (1960a), Lester (1963b), and Maini and Coupland (1964).

Sex Ratio

A sex ratio of three male trees for each female tree was reported by Pauley and Mennel (1957), after sampling one small population of seed-origin quaking aspen trees growing along a road fill in Minnesota. In Wisconsin, Einspahr (1960b) forced trees into flowering by girdling, in the summer, those individuals without sex buds. In a separate study in Wisconsin, Lester (1963b) arrived at the same conclusion as Einspahr, that there was no significant deviation from a 1:1 sex ratio between male and female trees in natural populations.

In a study of nursery-produced seedlings, D.W. Einspahr (unpublished) examined the field-planted progeny of 28 quaking aspen crosses made between 1956 and 1959. The earliest flowering occurred when the 1-year-old seedlings had been in the field for 6 years. In 8 of the 28 crosses, all surviving trees flowered in 1 or more of the 8 years of study, but in only 1 of the 8 crosses was there a significant deviation from a 1:1 ratio of male to female trees. Among all crosses, the frequency of flowering in all of the 8 years was 12 percent for female trees and 33 percent for male trees. All male and female trees flowered at least 3 years, and 61 percent of the females and 76 percent of the males flowered 6 of the 8 years. These data confirmed the more frequent flowering of male trees, and field observations indicated that male trees had more flower buds during the years that they flowered than did female trees.

Cut-Branch Technique

Branches with flower buds are collected between January and March and are forced in the greenhouse (Wettstein 1949). This has permitted extensive hybridization between local aspen species, as well as the exchange of aspen pollen between workers throughout the world. High yields of quaking aspen pollen are possible by collecting male branches from the upper crowns in February, usually by shooting off one main branch, removing smaller branches 100 cm or longer, and keeping their trimmed bases in water in an isolation chamber in the greenhouse (Einspahr and Benson 1964). Large male buds (fig. 5, A) soon elongate, and after 7 to 10 days at 18° C the male catkins are collected (fig. 5, B). The pollen is extracted, screened through a sieve with 2-mm pores, and stored over calcium chloride at 4.5° C. A number of different pollen sources can be handled in one greenhouse by the use of isolation chambers.



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Figure 5.—Cut-branch technique for hybridizing aspens: *A*, Newly-collected flower buds; *B*, pollen shedding; *C*, pollination of receptive female catkin.

Female branches are collected in March and forced in the same manner. When the red stigmas appear, pollen is applied with a small brush (fig. 5, *C*) and the branches are returned to isolation chambers and kept with their bases in ice water. Using this technique, production can average 150 to 300 seeds per catkin, with normal yields of half a million seeds per year.

Seedling Production

Aspen seeds are very small and wash away easily in nursery beds, and survivors are usually attacked by damping-off fungi and other pathogens. For small experimental lots, seeds are germinated in moist sand in the greenhouse and transplanted to indi-

vidual pots. However, the time and cost of this method is prohibitive when dealing with thousands of seeds, and excellent survival is routine when seeds are started in small covered nursery beds.

Seeds are sown on fumigated sand and covered lightly with additional sand. Watertight covers are placed over the beds and watering is done by hand for the first 2 weeks. For the next 4 to 5 weeks, the beds are covered with screens and watering is done with an overhead sprinkling system. Up to 150 seedlings per square meter (14 per square foot) are grown during the summer, measured and lifted in the fall, bundled, cut back to 30 cm and heeled-in overwinter in sand for spring outplanting (Benson and Einspahr 1962; Einspahr and Benson 1964).

ASEXUAL REPRODUCTION

Natural Suckering

The most common method by which quaking aspen reproduces itself in the field is by suckering from lateral tree roots (Sandberg and Schneider 1953). Vegetative shoots arise from adventitious buds on roots that are frequently less than 2 to 3 cm in diameter and growing within a few centimeters of the soil surface. In this manner, one seedling can propagate itself vegetatively to form a clonal stand

composed of genetically identical trees. A forest is thus often made up of adjacent stands of a dozen or more trees each, which are genetically identical within each stand but may vary greatly between stands (Pauley 1949). If left undisturbed, one clone can theoretically perpetuate itself indefinitely by root suckers.

Other types of natural suckering include occasional sprouting from stumps and root collars (Baker 1918) following the harvesting of young trees up to sapling size (Maini 1968).

Rooting

Air Layering

Air layering has not proven successful in the few known attempts made with quaking aspen (Chouinard 1956; Chouinard and Parrot 1958).

Stem Cuttings

The rootability of quaking aspen dormant stem cuttings is very poor compared to the relative ease of rooting other species of *Populus* (Maini 1968). Two exceptions have been reported, suggesting differences among clones in rooting. Snow (1938) collected 1-year-old cuttings in March, just when leaf buds were swelling. The bases were cut on a slant and soaked in 10 ppm (0.001 percent) IBA (indolebutyric acid) for 27 hours before planting in coarse sand, and 65 percent rooted. Barry and Sachs (1968) collected dormant stem cuttings in California and Baja California, and tested rooting after storage for periods of 6 and 12 months at three temperatures in the light and three in the dark. Treatments included quick basal dips in 0–4,000 ppm IBA or basal dips into Hormodin, a commercial rooting powder containing IBA. None of the California cuttings rooted under either field planting or greenhouse conditions. All cuttings collected from Baja California in April rooted. For other months, rooting for Baja material varied from 10 percent among cuttings collected in October to 96 percent for the June collection.

Root Sprouts and Sucker Cuttings

C. Muhle Larsen (1943) developed the technique of propagating root sprouts by planting isolated root sections of European aspen in moist peat in an unheated greenhouse. Root sprouts were excised when 3 to 5 cm tall and were rooted as greenwood or succulent cuttings. Johnsson (1953) used this method successfully in Ekebo, Sweden for many years, and it is now a common technique in many countries (Syrach-Larsen 1956). Research has shown that pathogen attacks can be reduced by collecting roots 2 to 3 cm in diameter in the fall after a hard freeze, scrubbing in water, and cutting to 150-cm lengths. Root sections are then dipped in a Captan solution (made with 1.5 tablespoons of Captan 50W per gallon of water), both ends are sealed with wax, planted in a sterilized 1:1 sand and vermiculite mixture, and watered "on the dry side" (Benson and Schwalbach 1970). Root sprouts should be excised when 3 to 5 cm tall and rooted in the same 1:1 mixture, in a flat dish covered with a plastic bag.

The term *suckering* specifically means the growth of adventitious vegetative shoots from lateral roots of trees. However, this term has been misused in

recent years to also describe the sprouting from severed root sections. In order to reduce confusion in the future, we suggest that the term *suckering* be confined to sprouting from roots still attached to the parental tree, and the term *root sprouts* be used for vegetative shoots grown from roots after they have been severed from the tree and planted either in the field or in the greenhouse. This would immediately identify the origin of the cutting, either from suckers on attached roots or from root sprouts on severed roots. This terminology will be used to describe all of the following work, even though the terminology of some authors may be different.

Maini (1965) found that the optimum length of root section for the maximum number of root sprouts was 10 cm. Severed root sections were planted in the field and in the greenhouse, and most of the root sprouts grew from that end of the section nearest the tree before it was severed (Maini 1968). Root sections were also planted in sand flats held at different constant temperatures. The frequency of root sprouts per section steadily increased at successively higher temperatures of 15° C, 18° C, and 24° C, but progressively decreased at further high temperatures of 31° C and 35° C (Maini and Horton 1966). Considerable variation was also reported in the number of root sprouts initiated from root sections collected from different clones (Maini 1967).

Farmer (1963) collected cuttings from root sprouts raised from root sections in the greenhouse, as well as from natural field suckers in Michigan. The bases of the cuttings from both sources were soaked in 0, 20, 50 or 100 ppm IBA for 24 hours before planting in sand. The rooting of root-sprout cuttings was about 90 percent, either with or without IBA. However, the rooting for the natural suckers for each test solution was, respectively, 27, 54, 62, and 72 percent. IBA thus stimulated rooting of sucker cuttings but not root-sprout cuttings of quaking aspen.

Zufa (1971a) severed root sections from quaking aspen, bigtooth aspen, and several aspen hybrids at different times of the year. Root sections, collected from one 12-year-old tree in the fall, produced 40 percent more root sprouts than sections collected from the same tree in the spring. Cuttings were isolated from root sprouts 2 to 10 cm tall and planted in individual plastic tubes. For quaking aspen, 85 percent of the fall-produced cuttings rooted, compared to only 66 percent of those produced in the spring.

Hicks (1971) compared the cellular morphogenesis of rooted root-sprout cuttings with that of stem cuttings, and also developed a bioassay for testing the rooting effects of exogenously-applied growth regulators to the base of root-sprout cuttings (Hicks 1972). Starr (1971) also collected lateral roots from quaking aspen, and found that the frequency of

root-sprout initiation was reduced after 8 months storage and completely eliminated after 15 months in the dark. Schier (1972), on the other hand, found that the total number of root sprouts per root cutting could be increased by removing the apices from early root sprouts, thus reducing the effects of dominance.

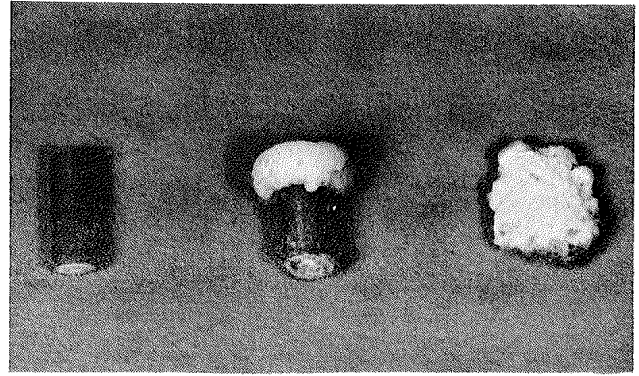
Grafting

Strothman and Zasada (1957) cited unpublished data from Pauley, who grafted quaking aspen scions onto short, unrooted stem cuttings of balsam poplar and willow. The lower cutting rooted first and sustained the upper scion, until it too rooted and could be separated and grown independently. Quaking aspen buds were successfully grafted onto quaking aspen, bigtooth aspen, and willow root stock (Pauley 1948). Sheat (1953) grafted quaking aspen scions onto gray poplar, and Einspahr and Benson (unpublished) successfully grafted quaking aspen to all species listed above, as well as to European aspen, white poplar, balsam poplar, and the hybrid *Populus x jackii* (*P. deltoides* x *P. balsamifera*).

For dormant scions, survival is higher if 1-year-old wood is used rather than older material. However, scions up to 6 years old can be grafted with almost as much success as 1-year-old scions by using bottle grafts described by Nienstaedt *et al.* (1958). This method is particularly useful for transferring slow-growing upper-crown flower scions onto potted root stock, followed by flowering 3 to 4 years after the grafts are established in the field. A modified crown-veneer graft is also useful for propagating mature scions onto 1-year-old root stock.

Tissue Culture

When a tree is wounded, new callus tissue quickly grows from the cambial cells to cover the opening. In a similar manner, if small sterile explants of cambial tissue are removed from tree trunks or seedlings, cambial cells will grow out from under the bark into a mass of undifferentiated tissue called callus. This derived callus tissue may then be isolated from the parental explant and grown independently on sterile nutrient medium made rigid by the addition of agar (fig. 6). After 3 to 6 weeks, the callus mass has usually exhausted the nutrients and must be cut into small cubes 3-mm square (subcultured) and grown on fresh medium. In this way a callus culture can be maintained indefinitely in the laboratory. If the callus tissue is very soft and loosely held together (friable), the cells will dissociate from each other in agitated liquid medium and form a cell suspension. Callus and cell cultures started from herbaceous plants and tree species have been used to study many problems of physiology, biochemistry, and pathology (Carew and Staba 1965; Lampert 1970).

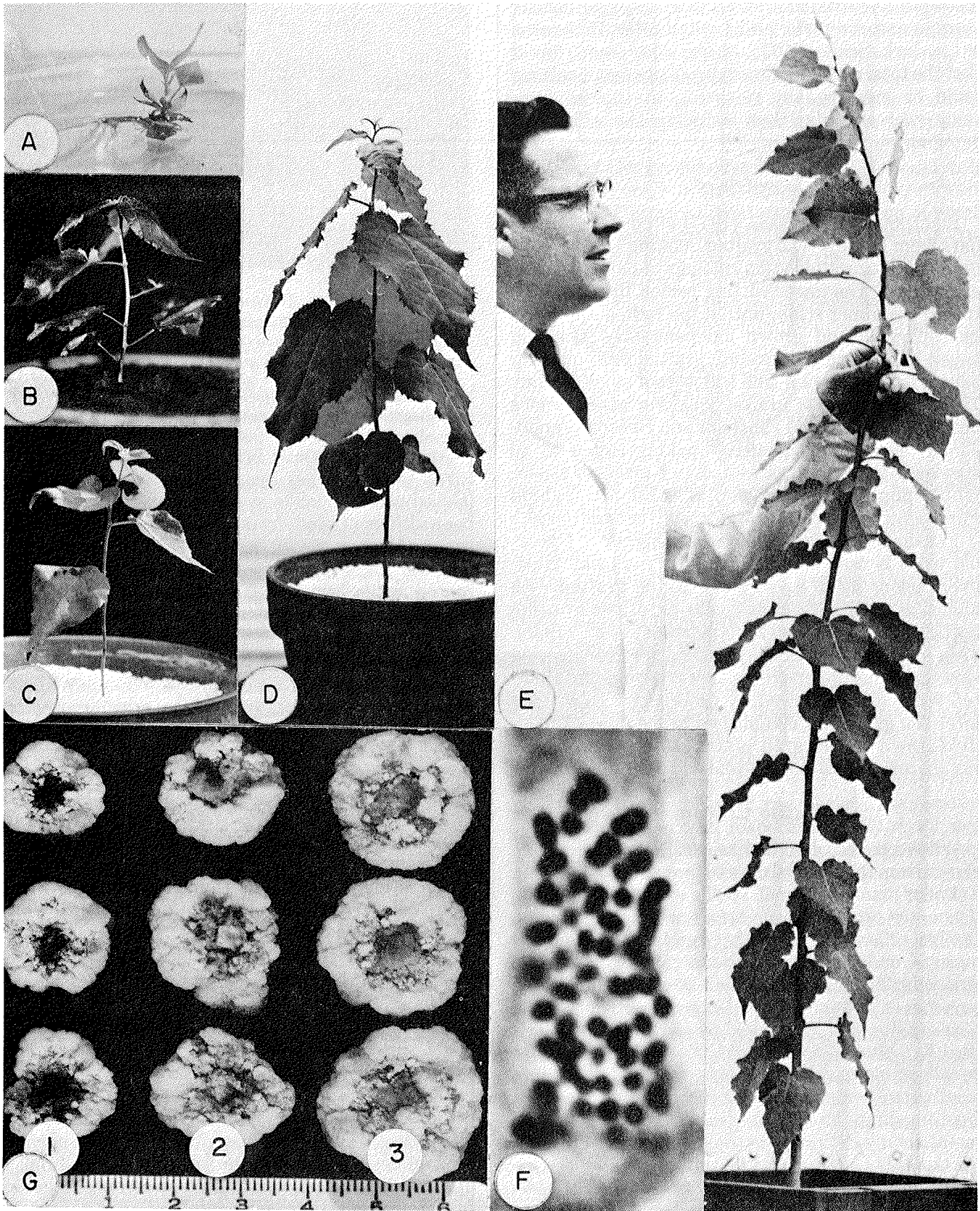


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Figure 6.—Initiation of callus. Sterilized internodal stem segment from triploid quaking aspen (left) placed on solidified nutrient medium. Firm white callus growing from the basal end of the segment, after growing for several weeks in the dark with the apical end in the medium (center). Isolated callus mass (right) growing independently on the medium (Mathes 1964a).

Another potentially important use of tissue cultures is the clonal propagation of tree species (Geissbuhler and Skoog 1957). Leafy shoots have been differentiated from callus tissue of quaking aspen (Mathes 1964b; Winton 1966; Wolter 1968), and the excised shoots rooted. Shoots have also rooted while still attached to the callus (Winton 1968b), and complete plants have been isolated and grown into normal trees (Winton 1970, 1971). On September 24, 1968, inocula from a triploid quaking aspen callus were transferred to a medium without auxin. A shoot grew after 3 weeks, which rooted on the callus after another 3 weeks. Figure 7, A–E shows growth of this complete plant 6 weeks after the root formed and 4 weeks after isolation from the callus. The tree is successively shown at 11 weeks; 14 weeks; 17 weeks; and 25 weeks, just before it was planted outside. Figure 7, F shows a normal triploid cell from a tree reproduced from tissue culture which is identical to cells from triploid tree. Firm white callus tissue is shown in figure 7, G, growing on different nutrient media just before transfer to shoot-initiation medium. Trees eventually developed from the fast growing callus of figure 7, G (3).

Unfortunately, this method of reproducing trees is not yet commercially feasible. Furthermore, the eventual efficient clonal propagation of trees will probably be from cell suspensions, in which each cell is turned into an embryo which develops into a plant by processes similar to those observed in the normal seed embryo. Further in the future is the possibility of removing the cell wall from tree-cell protoplasts, fusing protoplasts from remotely related genera or families, and growing a somatic hybrid capable of reproduction. This has only recently been done for any plant, using two closely related tobacco species (Carlson *et al.* 1972).



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Figure 7.—Propagation from tissue culture: *A*, Six weeks after root formed, 4 weeks after isolation from the callus; *B*, at 11 weeks; *C*, 14 weeks; *D*, 17 weeks; and *E*, 25 weeks. *F*, One triploid leaf cell from callus tree. *G*, Slow (1), medium (2), and fast (3) growing callus.

GENETICS AND BREEDING

Natural Variation and Inheritance

Important to satisfactory progress in tree improvement is reliable information on genetic control and natural variation. Both morphological characteristics and wood properties of quaking aspen have been extensively investigated. The clonal nature of quaking aspen has made it possible, by comparing within-clone and between-clone variation, to estimate genetic and environmental influence and obtain approximate estimates of broad-sense ³ heritability.

Growth, Form, and Wood Properties

Morphologically, quaking aspen is extremely variable and this variability has been the subject of considerable research. Variation in leaf morphology of naturally occurring clones has been described by van Buijtenen *et al.* (1959), Brown and Valentine (1963), Einspahr *et al.* (1963), and Barnes (1969).

³ The terms broad-sense and narrow-sense heritability are used to indicate the degree to which a character is influenced by heredity. Narrow-sense heritability is the fraction of total variation that is contributed by the additive effects of genes while broad-sense heritability includes both additive and nonadditive effects.

Considered were such characteristics as blade width, blade length, petiole length, number of serrations per centimeter, leaf tip angle, leaf base angle, and leaf shape. Moderate to strong genetic control appears to exist over leaf morphology as indicated by the broad-sense heritabilities reported by the above authors and tabulated in table 1.

Marked differences have also been reported in the flushing of aspen clones (Cottam 1954; Egeberg 1963; Strain 1966; and Barnes 1969) and differences in flushing have been reported to be useful in distinguishing clones. The work of Egeberg and Strain indicate a strong positive correlation exists between early flushing and frost injury.

Considerable variation has been encountered in bark color, thickness and texture (Cottam 1966), with Barnes (1969) and van Buijtenen *et al.* (1959) stressing the usefulness of bark characteristics in identifying aspen clones. Similarly, fall leaf color has been suggested by Barnes (1969) as a method of identifying aspen clones in Lower Michigan.

Variation in growth rate (height, diameter, tree volume, and crown volume) has been of considerable interest because of the economic implications. Large variations in growth measurements of quaking

Table 1.—Broad-sense heritability estimates

Characteristic	Data source			
	Barnes (1969)	van Buijtenen <i>et al.</i> (1959)	Einspahr <i>et al.</i> (1963)	Brown and Valentine (1963) ¹
Blade length	0.81	0.38	0.56	—
Blade width	0.80	0.51	0.69	—
Leaf shape	0.86	—	—	**1
Petiole length	0.82	0.29	0.82	**
Serration/cm	—	0.44	0.43	—
Leaf base angle	—	0.64	0.61	**
Leaf tip angle	—	0.39	0.87	**

¹ Broad-sense heritability values were not calculated. However, highly significant differences between clones were obtained for the characteristics indicated.

Table 2.—Broad-sense heritability estimates

Characteristic	Data source				
	van Buijtenen <i>et al.</i> (1959)	Barnes (1969)	Einspahr <i>et al.</i> (1963)	Einspahr <i>et al.</i> (1967a)	Einspahr & Benson (1967) ¹
Height	0.52	0.45	0.33	0.69	**1
Diameter	0.14	0.36	—	0.45	**
Tree volume	0.14	—	0.77	—	**
Crown volume	—	—	0.62	0.42	**

¹ Broad-sense heritabilities were not calculated. However, highly significant differences between clones within stands were obtained for the characteristics indicated.

aspen were reported by Barnes (1969) between 20 clones growing in Lower Michigan; by van Buijtenen *et al.* (1959) working with 20 clones in northern Wisconsin; and by Einspahr *et al.* (1963); and Einspahr and Benson (1967) investigating the variation between 4 clones of triploid and 75 clones of diploid quaking aspen in Wisconsin and Upper Michigan. Summarized in table 2 are estimates of broad-sense heritability calculated by these authors, along with information on twenty-five 5-year-old quaking aspen progeny groups (Einspahr *et al.* 1967a). Despite considerable variation in the heritability estimates, these data suggest moderate to strong genetic control over height growth and crown volume. Moderate control appears to exist over diameter growth and tree volume.

Specific Gravity

Specific gravity has been of research interest for a number of years, because of its importance to pulp yield, pulp quality, and the strength of structural timbers. Among 20 quaking aspen clones, van Buijtenen *et al.* (1959) found only a moderate amount of difference in the natural variation between clones, which, coupled with a fairly high within-clone variation, resulted in a broad-sense heritability estimate of only 0.17. Valentine (1962) sampled 23 areas in the Adirondack Mountains in a similar study. Although area means ranged from 0.343 to 0.432, a low broad-sense heritability (0.17), based upon intraclass correlations, was also obtained. Large between-tree variation within sampled areas was suggested as the reason for the low heritability. Brown and Valentine (1963), in an intensive study on four quaking aspen clones, obtained large within-tree variation in specific gravity, large within-clone variation, and highly significant between-clone differences, suggesting low to moderate genetic control. In a further intensive study of the pulp and wood properties of five quaking aspen clones, growing on fairly uniform site conditions in northern Wisconsin, van Buijtenen *et al.* (1962) calculated broad-sense heritability estimates for specific gravity of 0.43 to 0.58. Similarly, Einspahr *et al.* (1963), in a study on the wood and pulping characteristics of triploid quaking aspen clones, estimated the broad-sense heritability for specific gravity to be 0.38. Einspahr *et al.* (1967a) obtained an estimate of narrow-sense heritability of 0.42 from twenty-five 5-year-old full-sib quaking aspen progeny groups.

Geographic variation of specific gravity of quaking aspen has also been examined to a limited extent. Valentine (1962) found the five areas with the highest specific gravity occurred along the western periphery of the Adirondacks at altitudes of less than 384 meters (1,260 feet). Einspahr and Benson (1967), in a study of the geographic variation of

quaking aspen in Wisconsin and Upper Michigan, found a well-defined north-to-south trend of increasing specific gravity.

Obtaining broad-sense heritability estimates, using the clonal approach, requires that all trees within each clone be members of that clone and that care be exercised in using appropriately located wood samples which are free of stain, decay, and reaction wood. In the studies reviewed, where limited numbers of clones were employed and the trees were intensively studied, broad-sense heritabilities were higher than in studies that were of a more "extensive" nature. We are of the opinion that more reliance should be placed on the intensive studies even though more restricted populations were involved. In summary, the evidence to date, although variable, suggests that moderate to strong environmental control, and low to moderate genetic control exist over quaking aspen specific gravity.

Fiber Length

The fiber length of hardwood pulps has a considerable influence on pulp strength, particularly burst and tearing strength. Fiber length of quaking aspen, because it is relatively short (0.8 to 1.1 mm), has been the object of considerable research. J. P. van Buijtenen *et al.* (1959), working with clones of native quaking aspen growing in northern Wisconsin and in a more intensive study on five of the same clones (1962), obtained fiber length broad-sense heritability estimates of 0.35 and 0.51 for increment core samples, and 0.56 for fiber length when the measurements were made on pulp samples. Valentine (1963) and Brown and Valentine (1963) reported considerable between-clone variation in an investigation of four quaking aspen clones, but their data provided no valid heritability estimate. Einspahr and Benson (1967), investigating the variation in "age-30" fiber length of 225 quaking aspen (75 clones), obtained highly significant differences between areas, between clones within stands, and between stands within geographic areas, but failed to obtain any well-defined geographic trend in fiber length. They also found fiber length was positively correlated with height, diameter growth, and site factors affecting tree growth. They concluded that local site quality and genetic differences had an overriding influence on geographic trends in fiber length.

Triploid quaking aspen trees were reported (van Buijtenen *et al.* 1958) that had 26 percent greater fiber length than nearby normal diploid quaking aspen. In a study of four triploid clones growing in Upper Michigan, Einspahr *et al.* (1963) obtained broad-sense heritability estimates of 0.50 and 0.86 for increment core average fiber length and for "age-30" fiber length. Slightly lower broad-sense (0.58) and narrow sense (0.52) heritabilities were

obtained from a full-sib progeny test involving 25 progeny groups (Einspahr *et al.* 1967a). Fiber length in this study was also reported to be positively correlated with height and diameter growth.

In summary, considerable variation in fiber length has been encountered and the heritability estimates indicate moderate to strong genetic control. Considerable evidence exists that fiber length is correlated with rapid growth and that selection for improved growth rate can proceed hand in hand with improvement of fiber length.

Wood and Pulp Properties

Extensive use of quaking aspen as a pulpwood species has prompted investigations into the natural variation and genetic control of such wood and pulp properties as holocellulose, lignin, extractives, fiber strength, bursting strength, tear factor, and tensile strength. Clermont and Schwartz (1951, 1952) reported that quaking aspen had the highest holocellulose (80.3 percent) and the second lowest lignin content (18.1 percent) of eight hardwoods and eight conifers commercially important in Canada. Browning (1963) also indicated that quaking aspen has the highest glucan (57.3 percent) and the lowest lignin content (16.3 percent) of the 9 hardwoods and 10 softwoods listed. Similarly, Worster and Sugiyama (1962) found low lignin in both quaking aspen and black cottonwood.

Wilde and Paul (1959) found no significant difference in the chemical composition of quaking aspen growing on a wide variety of soils. Einspahr *et al.* (1967b), in addition to describing lignin levels similar to those given above, summarized the variation encountered in pulp yield, extractives, and fiber strength (zero-span tensile strength) of quaking aspen and quaking aspen hybrids.

Relatively little information has been published on the genetic and environmental control of wood and pulp properties. In whole tree pulping studies, involving 5 diploid quaking aspen clones (25 trees) and 4 triploid quaking aspen clones (20 trees), van Buijtenen *et al.* (1962) and Einspahr *et al.* (1963) obtained highly significant between-clone differences. They also presented variance ratios that indicated fairly strong genetic control over one or more fundamental fiber properties which, in turn, influenced such handsheet properties as bursting strength, tear factor, and tensile strength (broad-sense heritability estimates ranged from 0.50 to 0.90). Einspahr *et al.* (1963), in a previously cited study involving 25 full-sib progeny groups, obtained broad-sense heritability estimates of 0.58, 0.87, and 0.63 for lignin, extractives, and pulp yield, and interpreted the results as indicating moderate to strong genetic control over these properties.

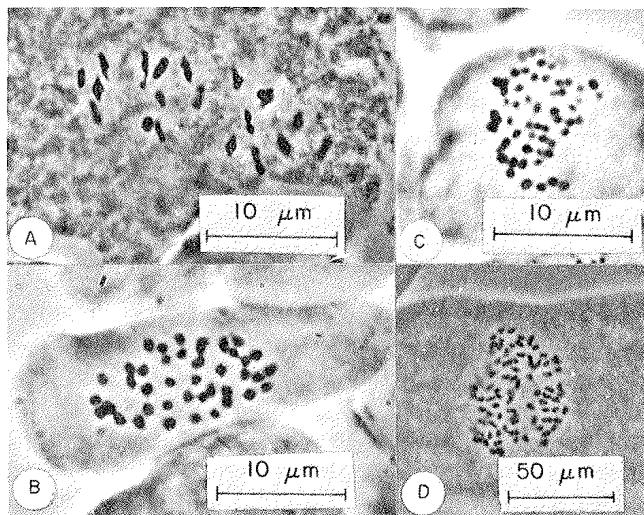
CYTOGENETICS

Chromosome Numbers

The haploid number of chromosomes for quaking aspen is $n = 19$ and the diploid number is $2n = 38$ (Erlanson and Hermann 1927). Thus, each newly formed cell in a normal diploid tree contains two sets of chromosomes. Preceding sexual reproduction, both male and female gametes (sex cells) are formed that normally have one-half the diploid number, which is one set of chromosomes per gamete. After the union of one male and one female gamete (fertilization), the diploid condition is restored for the growth of the new individual. Occasionally, the number of chromosomes is not reduced by half in the gametes, so seedlings may be found having three sets of chromosomes (triploids) per cell. Also, completely unreduced gametes from triploid trees may account for occasional tetraploids, with four sets of chromosomes. However, polyploid individuals are rare, and have only been reported in a half-dozen species of *Populus* (Darlington and Wylie 1956). The haploid number of all other reported species of the genus *Populus* is also $n = 19$ (Blackburn and Harrison 1924).

Figure 8, A shows 19 bivalents in metaphase I of

meiosis, from a male flower of quaking aspen squashed in propiono-orcein. Figure 8, B, 8, C, and



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Figure 8.—Quaking aspen chromosomes: A, Nineteen bivalents shown in metaphase I; B, leaf squashes show chromosomes averaging $1\mu\text{m}$ in diameter in a diploid cell ($2n = 38$); C a triploid cell ($3n = 57$); and D, a tetraploid cell ($4n = 76$). Each set contains one large and 18 small chromosomes.

8,*D* shows cells from the basal curl of 1-cm long expanding leaves of quaking aspen seedlings having diploid ($2n = 38$), triploid ($3n = 57$), and tetraploid ($4n = 72$) chromosome numbers. These squashes were prepared according to van Buijtenen (1959) as modified by Winton (1968c).

In most organisms, the genes which control sex are thought to be distributed among several chromosomes, but the expression of male or female characteristics is generally controlled by the balance of normal chromosomes (autosomes) and X (female) or Y (male) sex chromosomes. Peto (1938), Smith (1943), and van Buijtenen and Einspahr (1959) have reported what look and act like sex chromosomes in aspens, including quaking aspen. However, their results were not consistent for all trees studied, and this remains a controversial subject worthy of further investigation.

Triploidy

In 1934, H. Nilsson-Ehle (1936) found in Sweden a triploid European aspen (*Populus tremula* forma *gigas*), as a modified large-leaved tree in the forest which had three sets of chromosomes (Muntzing 1936). Johnsson (1940, 1955) eventually found natural triploids in about 20 areas of Sweden. This led to a search in the United States for triploids of quaking aspen, and as of now there are about one dozen known clonal stands in the Lake States Region (van Buijtenen *et al.* 1957). Most of the triploids were first identified in the field by their unusually large size and large leaves and were confirmed by chromosome squashes from newly-emerging leaves (van Buijtenen 1959; Winton 1968c). However, one of the early triploid clones was first identified by its unusually long fibers rather than by leaf size (J. P. van Buijtenen, personal communication).

Muntzing (1936) suggested that natural triploidy was produced by the fertilization of a normal female egg cell (haploid) by an unreduced male gamete (diploid) resulting in a triploid zygote. Cytological examination of pollen from normal diploid trees shows occasional large grains among the more abundant smaller and uniform grains. The largest size pollen may contain a completely unreduced chromosome complement of two sets.

Natural triploid quaking aspen have been reported that have longer fibers and greater volume than nearby diploid trees (van Buijtenen *et al.* 1957). Hence, polyploidy has been of interest as an improvement tool for aspen. Polyploidy programs have been in progress for many years in both Europe and the United States for hardwood species, and one of the objectives of aspen improvement has been the mass production of triploid progeny by sexual reproduction. Triploid organisms cannot produce sex gametes having even-numbered chromosome sets,

so they are either infertile or will not breed true. However, pollen grains from certain kinds of tetraploid male parents may contain two sets of chromosomes and can be used to fertilize normal female flowers, resulting in triploid seed. This means that an intermediate objective is the production of such tetraploid parents.

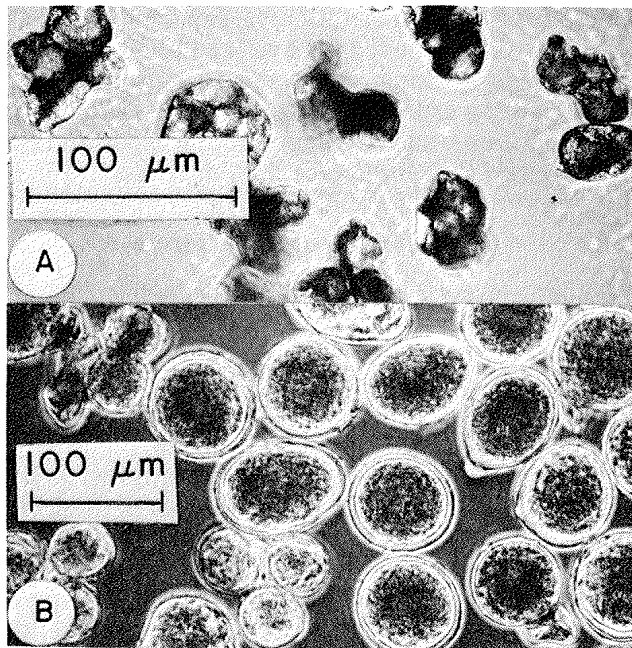
Tetraploidy

Mattila (1961) produced a tetraploid hybrid in Finland, by soaking swollen seed of *P. tremula* x *P. tremuloides* in 0.1 percent colchicine (Eigsti and Dustin 1955) for 1 hour before planting. In the United States, Joranson (1953) treated both ungerminated seeds and seedlings with colchicine solutions of 0.1 percent to 0.5 percent, but with only partial success. Trees with initial tetraploid chromosome counts later gave diploid counts, probably as a result of the incomplete doubling of each chromosome complement per cell by the colchicine treatment, followed by the eventual faster growth of the unchanged diploid cells. Einspahr (1965) treated newly-formed embryos with 0.3 percent colchicine for 6 hours, at 6-hour intervals after pollination. About one-third of the aberrant seedlings were tetraploid, but most of these reverted to diploidy during the following several years. A few, however, have remained tetraploid and are expected to flower soon.

In female flowers forced in the growth chamber (Winton 1968a), fertilization occurred about 24 hours after pollination, and the first mitotic division of the zygote took place after another 24 hours. This gave about 1 full day in which to treat the zygote with colchicine, in order to cause the single set of chromosomes to double in number at the first mitotic division. Doubling is accomplished by the inhibition of the fiber-spindle apparatus by the alkaloid colchicine, permitting the duplication of whole chromosomes without wall formation, thus producing instant tetraploidy. Numerous tetraploid seedlings were recovered from several such experiments, and the few which did not revert are still under study.

Another method of inducing tetraploidy is the use of unreduced pollen from a male triploid tree for pollinating normal females. The reason a triploid will not breed true is the impossibility of evenly distributing three sets of chromosomes among four haploid microspores during meiosis. The irregular chromosome distribution (aneuploidy) results in an array of pollen grains of different size, presumably correlated with the different amounts of chromosomal material received during meiosis. Assuming that the largest grains contained three full sets of chromosomes, large pollen was dry-separated on top of a sieve having pores of 37 mic-

rometer (fig. 9, A), then used to pollinate normal female flowers (Winton and Einspahr 1970). Figure 9, B shows unreduced pollen in water to indicate the approximate 20 percent increase in size when wet. Since reporting the first tetraploid quaking aspen tree produced by this method in 1969, a second similar tree was produced in 1972. Both trees are being propagated by grafting, but flowering has not yet begun. Figure 8, D shows a somatic tetraploid cell ($4n = 76$) from the tetraploid produced in 1968, presumably from the fertilization of a normal haploid egg cell with an unreduced triploid male gamete. A normal somatic diploid cell ($2n = 38$) is shown for comparison in figure 8, B.



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Figure 9.—Triploid quaking aspen pollen: A, Dry pollen showing one large unreduced grain among smaller pollen grains; B, wet pollen after sieving, with only the largest grains and undissociated tetrads remaining.

Monoploidy

A recent approach to aspen breeding is the production of haploid sporophytes (monoploids) for homozygous lines, selection of recessive markers, and cytological studies (Stettler and Howe 1965; Schreiner 1967). The most widely used method of producing monoploids of aspen has been the weakening of pollen with an agent that prevents fertilization but permits haploid embryo development of the unfertilized egg cell. Apparently, after normal fertilization, the zygote is stimulated into embryogenesis by a substance released or induced by the pollen tube entering the female gametophyte. Thus, the main objective in monoploid production is to inhibit gamete production, but still permit the pollen tube

to grow normally and trigger embryogenesis. If the active triggering substance turns out to be an auxin, then perhaps pollination by weakened pollen can eventually be replaced by applications of IAA. In this manner, the unfertilized haploid egg cell divides and grows into an embryo, perpetuating the haploid condition beyond the point where diploidy is normally restored by fertilization.

Monoploidy was first found in trees by Traulau (1957), in roots of a bushy European aspen. Kopecky (1960) produced monoploids of white poplar (*P. alba*), both by weakening European aspen by fermentation and by hybridization with black poplar (*P. nigra*). In the latter method, the pollen of black poplar (Section *Aegeiros*) probably stimulated embryogenesis without fertilization in the egg cell of white poplar (Section *Leuce*).

In the United States, monoploids have been produced in quaking aspen by Winton and Einspahr (1968) and Valentine *et al.* (1969), as well as in black cottonwood (*P. trichocarpa*) by Stettler and Howe (1965) and Stettler (1968). Stettler and Bawa (1971) demonstrated spontaneous doubling of the original haploid chromosome set (diploidization) in monoploid embryos. Spontaneous diploidization also occurred in one monoploid seedling produced by Einspahr (Winton and Einspahr 1968), resulting in the rapid growth of the new diploid branch.

Because of the strong tendency for spontaneous diploidization in quaking aspen, most workers have used male leaf markers and looked for aberrant diploid seedlings having maternal characteristics. Perhaps maternal progeny can never be proven to be homozygous diploids, but with repeated testcrosses to the female parent without the occurrence of male characters, the degree of doubt may be significantly reduced. One monoploid seedling grew to several inches in height but had male characteristics (L. L. Winton, unpublished data), indicating that it may have grown from a male gamete (androgenesis). However, this plant died after 1 year. Indications of androgenesis, followed by doubling, have been found, such as (1) chimeras having adjacent haploid, diploid, or tetraploid chromosome sets in smaller-than-normal cells, and (2) very strong leaf characters of the male parent. Unfortunately, in monoploid induction studies the incidence of aneuploidy is often high. Growth does not seem to be inhibited by these intermediate chromosome counts, but any traits of interest must then be further maintained by vegetative propagation. From one cross, using heat-treated pollen, three seedlings with intermediate counts of 40 chromosomes had characteristic multiple stems. Only more studies can show whether propagating these promising aneuploids may be of value. In addition, much more work is needed on all phases of monoploid production and utilization.

INTERSPECIFIC HYBRIDS

Natural and artificially produced hybrids are of interest because of the possibility of improved growth rate, climatic tolerance, wood quality, ease of propagation, resistance to disease and insects, and because of information such hybrids provide on biosystematic relationships within the genus. Quaking aspen, because of its ability to grow on a wide variety of soils and its extensive range, grows in close proximity to a number of other *Populus* species. Similarity in flowering habit, chromosome number, ease with which the crosses can be handled, and prolific seed production have made quaking aspens the subject of numerous investigations.

Natural Hybridization

Quaking aspen growing on sandy upland sites in the Lake States and Eastern forest regions are often found growing in intimate mixtures with bigtooth aspen. Between-species differences in time of flowering have been described as the principal reason large-scale natural hybridization does not occur between the two species. Victorin (1930) first reported and described a hybrid between quaking and bigtooth aspen. Heimburger (1936) produced hybrids by crossing the above species and attributed the scarcity of natural hybrids to the earlier flowering (7 to 10 days earlier) of quaking aspen. Pauley (1956) reported that quaking aspen flowered 10 to 14 days before bigtooth aspen in east central Massachusetts. Einspahr and Joranson (1960) observed similar differences in the time of flowering between quaking aspen and bigtooth aspen growing in northern Wisconsin.

Despite evidence on the differences in flowering times, there have been numerous reports of naturally occurring hybrids between quaking and bigtooth aspen. Pauley (1956) reported scattered hybrid individuals and hybrid swarms and attributed hybridization to the occurrence of temperature inversion situations in which quaking aspen female flowering was delayed to a point that it coincided with the flowering of nearby bigtooth aspen clones. Einspahr and Joranson (1960) reported evidence of "late flowering" female quaking aspen clones that, in addition to flowering at the normal time, produced numerous flowers approximately 10 days later. Such flowering appeared to coincide with the flowering of nearby bigtooth aspen. Barnes (1961), in a study of hybrid aspen in Michigan, reported 38 hybrid clones, the majority having leaf characteristics intermediate between the two parents. Barnes also reported evidence of "late flowering" and felt that interclonal variation in flowering time also contributed to the occurrence of natural hybrids. Andrejak and Barnes (1969) in studies on a seedling population

in Michigan, and Barnes (1967) working with western quaking aspen of the Columbia Plateau, reported additional evidence of hybridization between bigtooth and quaking aspen.

Natural hybrids between quaking aspen and several other species of *Populus* have also been reported. Heimburger (1936) and Peto (1938) reported the occurrence of hybrids between *P. alba* and quaking aspen in Canada, and P. N. Joranson (unpublished) and Barnes (1961) reported hybrids of similar origin growing in Iowa and Lower Michigan. Brayshaw (1965), in describing native poplars and their hybrids in southern Alberta, reported putative hybrids that he believed to have resulted from crosses between quaking aspen and *P. deltoides*, between quaking aspen and *P. x jackii* (*P. deltoides* x *P. balsamifera*), and between quaking aspen and *P. x andrewsii* (*P. tremuloides* x *P. deltoides*).

Artificial Hybridization

Quaking aspen, because of its economic importance and because of the ease with which it can be crossed with other species of *Populus*, has been widely used as a parent in experimental crossing research. Some of the earliest descriptions of the use of quaking aspen as a parent tree include reports by Wettstein (1933), Stout and Schreiner (1933), Heimburger (1936), and Johnsson (1953, in describing crosses he made in 1939). Pauley *et al.* (1963b, 1963c) described several *P. tremuloides* x *P. tremula* and *P. tremuloides* x *P. grandidentata* crosses made in 1950 and 1951 which apparently represent, along with the work of Stout and Schreiner, the earliest extensive production and testing of quaking aspen hybrids in the United States. Early successful crossing and rapid growth of *P. tremuloides* by *P. tremula* hybrids resulted in extensive trials employing quaking aspen in tree improvement programs in Asia, Europe, Canada, and the United States (Johnsson 1952, 1953; Syrach-Larsen 1953; Hyun and Hong 1959; Heimburger 1958, 1968; Einspahr and Benson 1964; Seitz 1963; Melchior and Seitz 1966; Hattemer and Seitz 1967; and Zufa 1969).

A review of the literature indicates quaking aspen has been successfully crossed with a large number of species of *Populus*, including species within the sections of *Leuce*, *Aigeiros*, and *Tacamahaca*. Table 3 summarizes the reported successful crosses along with remarks on the ease of crossing. Quaking aspen crosses readily within the section *Leuce* and with difficulty with species in the other sections of the genus *Populus*. Based upon growth rate and form, the most promising type of hybrids are those originating from crosses between quaking aspen and *P. tremula*, *P. alba* and *P. canescens*. Figure 10

Table 3.—Hybrids having quaking aspen parentage

Type of cross ¹	Ease of crossing	Remarks
<i>P. tremuloides</i> x <i>P. tremula</i> ^L	Easy	Very promising; reported by numerous workers; see literature cited in text
<i>P. tremuloides</i> x <i>P. gradientata</i> ^L	Easy	Some crosses promising; reported by numerous workers; see literature cited in text
<i>P. tremuloides</i> x <i>P. alba</i> ^L	Easy	Some crosses promising; reporting by numerous workers; see literature cited in text
<i>P. tremuloides</i> x <i>P. sieboldii</i> ^L	Easy	Moderate growth rate; several reports including Zufa (1968) and Einspahr & Benson (1964)
<i>P. tremuloides</i> x <i>P. davidiana</i> ^L	Easy	Moderate growth rate; reported by Heimburger (1958) and Einspahr & Benson (1964)
<i>P. tremuloides</i> x <i>P. adenopoda</i> ^L	Easy	Moderate to rapid growth; Heimburger (1958) and Pauley <i>et al.</i> (1963c)
<i>P. tremuloides</i> x <i>P. canescens</i> ^L	Easy	Promising crosses reported by numerous workers ²
<i>P. tremuloides</i> x <i>P. deltooides</i> ^A	Difficult	Slow growing hybrids; reported by Stettler (1968), Zufa (1968), The Institute of Paper Chemistry, ³ and University of Wisconsin ³
<i>P. tremuloides</i> x <i>P. balsamifera</i> ^T	Difficult	Zufa (1968)
<i>P. tremuloides</i> x <i>P. x jackii</i> ^{AT}	Difficult	Zufa (1968)

¹ The usual procedure is to list the female parent first. In most instances listed above, the reciprocal crosses have also been successfully completed. The superscripts indicate the section within the genus that the species is located, L = *Leuce*, A = *Aigeiros*, and T = *Tacamahaca*.

² Reported by numerous authors, including Heimburger (1936), Einspahr & Benson (1964), Melchior and Seitz (1966), and Hattemer & Seitz (1967).

³ Unpublished reports, data on file at the respective institutions.



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Figure 10—Thirteen-year-old triploid hybrid aspen (*P. tremuloides*, 2n x *P. tremula*, 4n) growing on a sandy site in northern Wisconsin. Total height averaged 15.5 meters (51 feet) with a breast height diameter of 15.2 centimeters (6.0 inches).

illustrates the outstanding form and growth rate obtained from triploid hybrid crosses (*P. tremuloides*, 2n, x *P. tremula*, 4n).

TREE IMPROVEMENT PROGRAMS

Forest tree improvement is usually considered to be synonymous with tree breeding and is the practical extension of forest genetics. In the Lake States Region, where aspen pulpwood is of considerable importance, the goals of quaking aspen tree improvement work include improvement of growth rate, wood quality, and disease and insect resistance. Available to tree improvement workers are such techniques as plus-tree selection, hybridization, mutagenesis, and polyploidy. Fortunately, in the case of quaking aspen, all four techniques can be expected to make valuable contributions to the goals cited above and, as will be discussed later, the greatest gains can be expected through the use of a combination of these methods.

Determining Tree Improvement Goals

Because of the long-term aspect of tree improvement, researchers must carefully consider the wood, growth, and morphological characteristics selected for improvement. Generally, it is agreed that the most useful and rapid approach to use is to select a limited number of characteristics and to carry on the improvement of all characteristics simultaneously. For each characteristic under consideration the

geneticist must know something about the natural variation, heritability, importance to the final product, and the interrelationships that exist between selected characteristics. Viewing quaking aspen in this light and considering previously cited information on natural variation, heritability, and wood and pulp properties, there appears to be little doubt that considerable improvement in growth, wood quality, and insect and disease resistance can be obtained through a properly oriented tree improvement program.

Realistically, a tree improvement program of any magnitude should be located in those areas where the species in question is an important component of the forest region. At present, The Institute of Paper Chemistry, Appleton, Wis., and The Ontario Department of Lands and Forests, Maple, Ontario (Zufa 1971b) have programs in which considerable emphasis is being placed on the improvement of quaking aspen. Valuable contributions to the knowledge regarding the genetics and silvics of quaking aspen have been made by numerous researchers, as is obvious by the many literature citations in the previously reviewed sections. Canadian research on quaking aspen has greatly increased during the past 10 years, as is evident by the coverage given by Maini and Cayford (1968) in "Growth and Utilization of Poplars in Canada."

Selection of Parent Trees

Selection, as a tree improvement technique, is rapid, inexpensive, and normally considered to be a first step in most tree-improvement programs. The principal disadvantage of the use of selection, to the exclusion of other methods, is that there is an upper limit to the expected gains. The best selections will be no better than allowed by the population gene pool being exploited. Although detailed selection standards for quaking aspen have not been published, they do not differ greatly from the standards established for conifer species. Rudolph (1956) published the earliest guideline for selecting superior trees in the Lake States Region and included a brief section on aspen. He emphasized the selection of trees free from insect and disease problems, with good stem form and fine branching, and with growth rates of not less than 70 centimeters (2.3 ft.) per year in height and not more than 1.8 rings per radial centimeter (4.5 growth rings per inch). Einspahr (1960b) briefly described an empirical selection index for quaking aspen based on tree volume/age, tree volume/crown volume, tree form with adjustments for excessive crown size, and site index. A selection index was developed by van Buijtenen⁴

⁴ Unpublished report on file at The Institute of Paper Chemistry, Appleton, Wis., 1960.

for quaking aspen which considered natural variation, heritability, and the economic value of the traits involved. Wood quality evaluation of quaking aspen parent trees has also been given appropriate consideration by Einspahr and Benson (1967) in a study which provided "base lines" for judging fiber length, specific gravity, and fiber strength of selected trees. Single-generation gains approaching 30 percent for volume growth, 10 percent for fiber length, and 6 percent for specific gravity can be expected through selection. Because of the difficulty and cost of vegetative propagation, it is expected that most selected quaking aspen trees will be used as parent trees rather than for clonal propagation.

Parent Tree and Progeny Evaluation

Provenance tests, which have been widely utilized in evaluating appropriate seed collection zones for many forest trees, have not been extensively utilized in tree improvement work on quaking aspen. Pauley *et al.* (1963a) described one of the few such studies in which quaking aspen seed sources were involved. His principal conclusion was that western high-altitude sources were unsuited for use in Massachusetts and were probably unsuited for use in the Lake States Region.

Much of the evaluation work with quaking aspen has involved evaluating full-sib progeny and clonal tests. Heimburger (1940) emphasized the importance of evaluating young hybrids for hardiness, disease resistance, and the ability to root from stem or root cuttings. Heimburger (1958) and Zufa (1969) discussed the evaluation of aspen hybrids for growth and rooting ability. Johnsson (1953) evaluated diploid and triploid *P. tremula* x *P. tremuloides* hybrids using small full-sib progeny groups. His results illustrated the rapid growth of triploid material and the superiority of both types of hybrids over the native aspen controls. Johnsson concluded that the hybrids could be expected to produce approximately twice the usable wood volume as the best native aspen.

Melchior and Seitz (1966) and Hattemer and Seitz (1967) described full-sib progeny tests of aspen hybrids, the majority of which were crosses having quaking aspen as the male parent. Their results appear to substantiate previous reports regarding the superiority of *P. tremula* x *P. tremuloides* hybrids. Similarly, Einspahr and Benson (1964) and Benson and Einspahr (1967) described replicated full-sib progeny tests involving diploid and triploid quaking aspen and triploid hybrid aspen. Highly significant differences were obtained between experimental materials for such properties as specific gravity, fifth-year height growth, diameter growth, and natural pruning. Additional measurements made on these progeny groups, to evaluate volume

growth (D. W. Einspahr, unpublished) and wood properties (Einspahr *et al.* 1970), indicated that by age 10 the triploid hybrids were growing approximately twice as fast (3.2 vs 1.4 cu m/yr), had 18 percent longer fibers, 20 percent higher specific gravity, similar levels of lignin and extractives, and improved paper properties.

Quaking aspen and quaking aspen hybrid progeny groups have also been evaluated for a number of other properties, including wood quality (Einspahr *et al.*, 1967a; Einspahr *et al.* 1968), nutrient requirements (Einspahr 1968), and disease resistance (Johnsson 1953; Heimburger 1940; Pauley 1963c). Improvement in resistance to hypoxylon canker (*Hypoxylon mammatum* or *H. pruinautum*) looks particularly promising through the use of selected resistant quaking aspen parent trees and through hybridization using species within the section *Leuce* which show natural resistance to hypoxylon. Recently acquired data (Einspahr and Benson, unpublished), from 10- and 15-year-old quaking aspen progeny groups, suggest that control crosses will have 15 percent killed or infected trees by age 10 and 30 percent hypoxylon by age 15. Some of the best *P. tremuloides* x *P. tremuloides* genetic combinations are expected to have only 10 to 15 percent hypoxylon by age 15.

Quaking Aspen for the Year 2000

Forestry research has long suffered from lack of foresight and long-range planning. Forest genetics research is no exception. Too much effort has been spent solving today's problems and not enough consideration has been given to how future harvesting methods, future wood and paper product developments, and predicted land use trends affect forest tree improvement goals. There are many individuals, the authors of this paper included, who feel that in the future there will be a greater emphasis on short rotations and the use of wood as a raw material for paper, paperboard, particle board, chipboard, and similar reconstituted products. Many of these same individuals believe that in order to remain competitive, there must also be major changes in harvesting procedures. "Chipping in the woods" without prior debarking is the approach being favored, especially for hardwood cellulose production. Land use trends will also eventually have an important influence on the type of improved materials required. Reductions in both site quality and total acreages available for forestry production suggest the need for genetically improved tree species that will do well on less productive soils and will respond to intensive forestry practices.

After working for a number of years in a research area that lies at the interface between forest genetics, pulp and paper technology, and woodlands

management, it is our opinion that a tree improvement program for quaking aspen should emphasize: (1) maximum per hectare per year cellulose production through improved height and diameter growth, improved form (natural pruning, straightness, and narrow crown), and vigorous suckering; (2) high juvenile wood specific gravity for greater pulp yield per cord and improved tearing strength; (3) longer fiber length in an effort to improve basic paper properties including tear, burst, and tensile strength; (4) improved insect and disease resistance with special emphasis on hypoxylon canker and wood borers of the genus *Saperda* and *Agrilus*; and (5) improved response to intensive forestry practices.

Quaking aspen tree improvement workers are fortunate in having available several methods of tree improvement, the most promising of which are selection, hybridization, and polyploidy. Greatest gains can be expected from the use of a combination of methods. It is proposed that an elite gene pool of diploid material first be developed, by using a recurrent selection and progeny testing system similar to that described by Schreiner (1970) for cottonwood. Then, using the improved diploid material, it would be possible to produce new genetic combinations using polyploidy, interspecific hybridization, and combinations of hybridization and polyploidy.

Gains from selection and first generation intra-specific breeding are expected to be 20 to 30 percent in volume growth, 2 to 5 percent in specific gravity, 5 to 7 percent in fiber length and 40 to 50 percent in hypoxylon resistance. Additional gains through the use of polyploidy and hybridization are expected to be nearly twice those obtained from selection and first generation breeding. Naturally occurring quaking aspen triploids, for example, consistently have had 25 to 30 percent longer fibers (van Buijtenen *et al.* 1958; Einspahr *et al.* 1967b) and recent 10th-year observations had only 3 percent hypoxylon, less than one-third the normal level of infection at age 10. Triploid hybrids involving crosses between *P. tremuloides* and *P. tremula* had a specific gravity of 0.42 at age 10 while comparable diploid quaking crosses had a specific gravity of 0.35.

Site requirement information (Zehngraff 1947; Stoeckeler 1948) and preliminary observations on the fertilization and irrigation of aspen (Einspahr *et al.* 1971) indicate that quaking aspen will respond to intensive management procedures. Form, natural pruning, and the number of stems per acre at ages 10 to 20 years make it ideally suited to mechanical harvesting techniques. Preliminary volume measurements (Einspahr and Benson 1970; Benson and Einspahr 1972) on young quaking aspen stands indicate that by capitalizing on rapid juvenile growth and improved utilization, annual per acre volume production can be approximately doubled.

Wood quality, always of concern when rate of

growth is rapid, does not seem to be impaired by factors influencing rapid growth rate. Pronin and Lassen (1970), in reviewing and evaluating the relationship between site quality and specific gravity of quaking aspen, found there was no consistent relationship and concluded that volume growth could be emphasized without concern for specific gravity variation associated with site quality. Considerable evidence also exists which indicates that there is a positive correlation between diameter and height growth and fiber length in young quaking aspen and eastern cottonwood. These results, when considered with available information on heritability of

growth characteristics, suggest that measures taken, either genetically or environmentally, to improve growth rate will result in improved fiber length.

Quaking aspen, particularly in the Lake States Region, is presently providing the region with a valuable resource. Properly oriented genetics and intensive management research programs stressing rapid growth, "juvenile" wood quality response to intensive management, and improved utilization can be expected to make aspen an even more valuable resource in the future.

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