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Field Guide to Aid in Recognition of Natural Triploid Aspen

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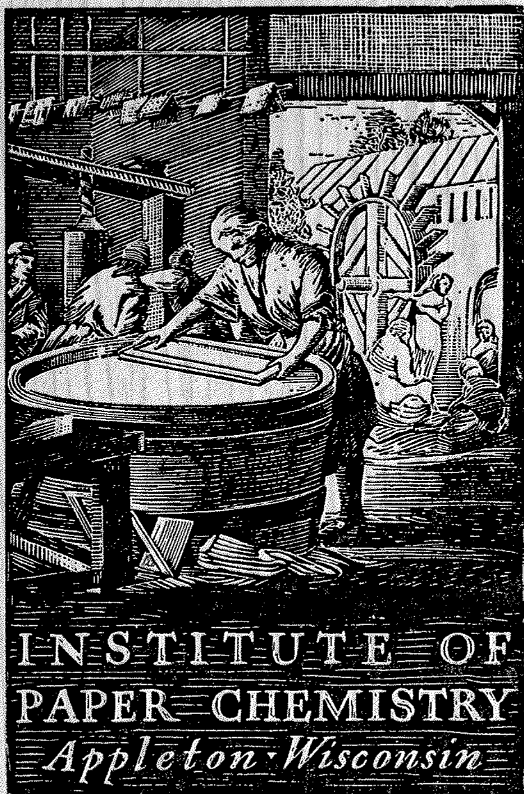

A FIELD GUIDE TO AID IN RECOGNITION OF NATURAL TRIPLOID ASPEN

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LAKE STATES ASPEN GENETICS AND
TREE IMPROVEMENT PROJECT

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By

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A. BACKGROUND

Swedish experience with the genetic improvement of the European aspen (Populus tremula L.), a species which is closely kin to our North American quaking aspen (P. tremuloides Michx.), suggests that the rate of volume increment and the length of fiber tracheids might both be increased in North American aspen by finding or producing trees which possess a triple set, rather than the usual double set, of "chromosomes". Chromosomes (Figure 1) are found within an inner region--the nucleus--of each of the millions of cells formed during the growth of a tree.

Arranged on the chromosomes, somewhat like beads on strings of varying length, are the ultimate heredity determiners, known as genes. Every aspect of the development of a tree--be it the structure of a fiber, chemical constitution, rate of growth or disease susceptibility--is under the guiding influence of a group of genes, each of which supplies some specific contribution to the developed characteristic.

The discovery in Sweden of a European aspen (P. tremula L.) of large size by Nilsson-Ehle (7) and the determination by Müntzing (6) that this tree possessed three sets (the triploid number) of chromosomes, rather than the normal double set (the diploid number), did much to speed the investigation of the usefulness of polyploidy in tree improvement. Johnsson (1, 5) has reported that, as the result of intensive searching, triploid aspens were located in some 20 areas ranging from southern to northernmost Sweden.

Fig. 1. The chromosomes of triploid (above) and diploid (below) aspen. Reproduced and enlarged from drawings of chromosomes in the root tips of European aspen (P. tremula L.), with the kind permission of Helge Johnsson (1). Magnification, ca. 5,000X.

The Swedish triploids drew attention because they were often larger trees than the neighboring diploids. Measurements made by Petrini (8) in a natural stand approximately 57 years old indicated that the triploids exceeded the diploids by 36 per cent in volume, 10 per cent in diameter and 11 per cent in height. In addition to the tendency to produce wood more rapidly, there were indications, reported by Johnsson (2), that, as might be expected on the basis of much evidence in non-tree species, triploid fibers were larger than diploid fibers. In one case where the triploid was an artificial one, the proportional increase in length was greater than the proportional increase in width. The latter result gave encouragement to efforts to lengthen the fibers of North American aspen without radically changing other aspects of fiber structure, and suggested one possible avenue of approach toward the industrially important objective of enhancing the strength of papers made from aspen.

Based in part upon these considerations, the program[1] of the Genetics Section of The Institute of Paper Chemistry has included an intensive search for triploid aspen in the forest, and a detailed study of its growth, wood quality, and pulping characteristics. Triploid quaking aspen clones have been found on the Superior National Forest near Forest Center, Minnesota, near Bruce Crossing in Eastern Upper Michigan's Ottawa National Forest (two groups) and near Crested Butte, Colorado. A number of bigtooth aspen (*P. grandidentata* Michx.) clones have been examined, but no triploid bigtooth aspens have been located.

A paper by J. P. van Buijtenen *et al.* (9) describes the triploid discoveries in some detail and discusses triploid-diploid differences in volume growth, morphology, and anatomy. Studies dealing with various aspects of wood quality and with the pulping properties of triploid *vs.* diploid aspen are also

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[1] The work described is a major feature of The Institute of Paper Chemistry Project 1800, in which a group of pulp and paper companies share support.

being carried out and will be reported in the near future.

More triploid aspens are needed, and the descriptions and suggestions which follow are offered as an aid in finding them in the forest. New discoveries will provide more material for testing and observation, and a better base for trials looking toward commercial production of triploid aspen for pulpwood.

B. TWO PROCEDURES FOR RECOGNITION OF POSSIBLE TRIPLOID TREES

1. The Large Leaf Method

Though it seems possible that there are occasional exceptions to the rule, it has been commonly observed that the leaves of naturally occurring triploid aspens are enough larger than those of neighboring diploid trees, so that the difference can be noted in the field. This was first reported to be the case with *Populus tremula* in Sweden, where the leaf size difference has been regarded as the most useful clue to triploid identification in nature (Johnsson 1, 3, 4). Recent observations and measurements of quaking aspen leaves taken from a mature stand in Upper Michigan in which a number of diploid and triploid stems occur together also support this generalization. Leaf samples from three diploid trees in this stand averaged 5.2 cm. in length and 5.2 cm. also, in width. Based on leaf measurements of four nearby triploid trees, average triploid leaf length was 6.2 cm. and leaf width, 6.4 cm. This average of 15 per cent difference (based on the diploid dimensions) in both length and width results in a proportionately greater increase in leaf surface area which is easily noticed from the ground. While there is some indication that this much of a difference does not invariably separate the two hereditary types wherever they occur together, such exceptions do not appear to detract much from the value of the leaf size method of searching for possible triploids.

Outlines of leaves showing the average dimensions found in this sample have been reproduced in Figure 2. The difference in size was established by measuring the third and fourth leaves back from a lateral twig's terminal bud, sometime after July 1, before which date leaf enlargement on lateral branches has been completed. Leaves in these positions were measured on 10 lateral twigs from one main branch sawed from the stem near the middle of the crown's length, on a side with good crown light exposure.

In searching for triploid trees, it is essential to recognize two other groups of causes which can produce leaves of large average size. The first group includes those genetic effects which are not due to the presence of an extra set of chromosomes. The second group includes nongenetic factors: age of the tree, manner of tree origin (sucker *vs.* seedling), immediate site conditions, disease, and various kinds of damage which result in a reduction in the number of buds. Thus, with both of these groups of causes frequently affecting average leaf size, it is quite apparent that diploid trees will vary so widely over a large forest area that some diploid stems may be found which have average leaf sizes as great as the leaf size on some of the triploids. *Eliminating from consideration any trees with large leaves traceable to the nongenetic causes mentioned above, however, there is little doubt that triploid leaves will nearly always be the largest ones in the local stand in which they occur.*

With a little practice, the effects of a number of nongenetic factors can often be recognized very quickly. In experience so far, probably three-fourths or more of the several thousands of aspens with large leaves which have been observed were rejected in the field as triploid suspects because it seemed more or less obvious that one of a group of nongenetic influences could have been responsible for the development of large leaves. These influences are described in more detail in the following:

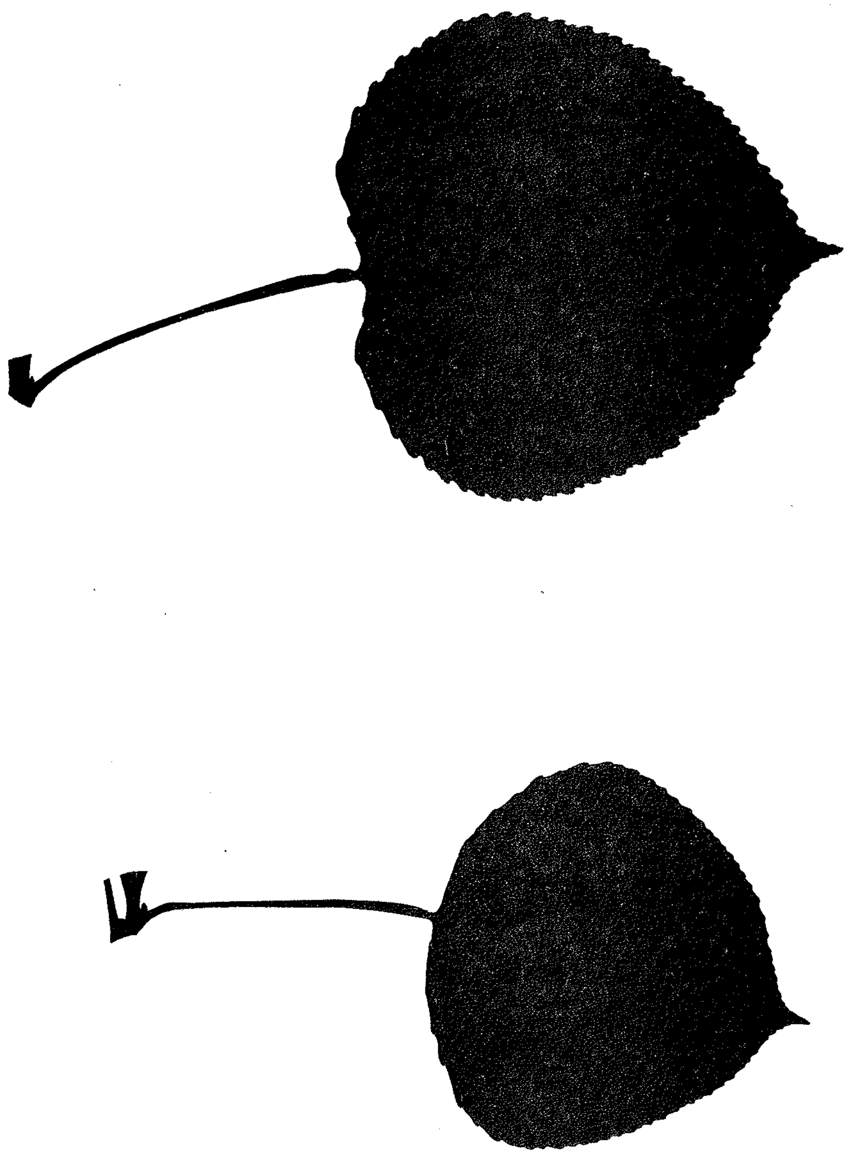

Fig. 2. Relative sizes of triploid (above) and diploid (below) leaves of naturally occurring quaking aspen, based upon leaf measurements from four triploid vs. three neighboring diploid stems, as described in the text.

a. On very young stems, origin as suckers (sprouts) from the root systems of older trees, _vs._ seedling origin. As a general rule, make few selections of possible triploids in stands less than 20 feet in height.

b. Especially favorable growing conditions localized in the immediate vicinity of the tree, or of a larger area within the stand.

c. Response to girdling, or to reduction of the number of buds from which leaves can develop, resulting in larger leaves from the live buds which remain.

   1) Girdling may be effected by rabbits or mice, or evidently _by the growth of an Hypoxylon canker on the main stem_, which has been observed very frequently on trees bearing large leaves. The apparent girdling effect of the _Hypoxylon_ canker seems to be one of the most common non-genetic causes of large leaf development.

   2) Reduction in the number of live leaf buds may result from feeding by deer or porcupines, from early frost and winter-killing, from extreme drought in the growing season--causing the leader to die back--and from storm breakage.

2. The Large Tree Method

A second method for detection of triploid aspens, and, as far as is known, a method original with the present project, is to look for exceptionally large veteran trees. If triploids in nature live as long as diploids and, as indicated by some data secured by Petrini (8) in Sweden and by van Buijtenen _et al._ (9) in Upper Michigan, reach greater diameters and heights, then among exceptionally large trees there may be a relatively high proportion of triploids.

This possibility has been checked with success in the current project. Almost all woodlands and forestry staffs remember the location of trees or stumps of unusual size. Such a recollection of a large aspen stump on the part of Mr. Herman Wickman, foreman for the Tomahawk Timber Company operation in the Superior National Forest in northeastern Minnesota led to a report of it by the company superintendent, Mr. Paul St. Amant. Chromosome counts from root tips and from young leaves have since shown that the tree reported was one of several triploid trees of similar age, all of which were cut, and which belong evidently to the same clone, since all are known to be triploid. The stump which attracted chief notice was from a tree which at an age of approximately 132 years had reached a stump diameter (at 20 inches height) of 24 inches, outside bark, and a height of approximately 95 feet.

Both standing trees and stumps of trees recently cut should be reported in pursuing this method, since stump roots and suckers still connected to the stump can be used for eventual determination of chromosome number and for vegetative propagation.

3. Additional Observations Regarding Both Methods

In applying both of the methods described, it should be kept in mind that triploid trees found in various locations will differ from each other in not a few characteristics, including some variation even in the "diagnostic" characters.

The large-tree method sometimes may be used in combination with the large-leaf method. Thus, in some situations the best candidates for marking in a stand of exceptional size may be the biggest trees with the largest leaves.

Also, when a single tree or clone having leaves of large size appears to be worthy of marking, it may be possible to obtain additional circumstantial evidence about the number of sets of chromosomes which it contains by observing certain other characteristics. Thus it is known that in some instances: (1) Triploid clones flush earlier in the spring than nearby diploid clones; (2) triploid leaves may be more darkly colored than the leaves of surrounding diploids; (3) triploid leaves may be somewhat wider than diploid leaves; (4) the teeth along the margin of triploid leaves may be larger; and (5) triploid flower buds and mature catkins may be bigger. Again, these are <u>not known to be consistent features</u>, but the fact that <u>some</u> triploids have shown one or more of these characters is of some obvious utility in refining the search for new ones.

C. REPORTING OF TREES JUDGED TO BE POSSIBLE TRIPLOIDS, AND FOLLOW-UP

1. Preliminary Marking and Description of Access and Location

When trees or stumps of trees cut recently are located by either of the two methods, they should be marked by any method which it is convenient to use, except that no suspect trees should be blazed. In addition to legal description, give compass bearing and distance from a point on a nearby road or trail or from a prominent landmark which can be found easily. Arrangements should be made to spare the trees marked, at least temporarily, until the chromosome count can be made.

2. Reporting

Information on the locations of trees marked as possible triploids should be sent to the Genetics Laboratory, The Institute of Paper Chemistry, Appleton, Wisconsin. Besides location, it should include report of method used, diameter at breast or stump height (when tree has been cut), and tree height. Any additional information will be welcome. As soon as possible, a member of the Genetics staff will arrange with the reporter to visit the tree or stump marked. In the interest of efficiency and economy, it is urged that where an active woods search is being made, reporting be delayed until several trees have been found which could be visited on the same trip out from Appleton.

3. Collection of Sample for Determination of Chromosome Number

Ordinarily, the sample taken from the tree for determination of chromosome number will be in the form of several short sections of root of about the thickness of a pencil. It is often not a simple matter to secure such a sample with full assurance that the roots taken are part of the root system of the tree to be sampled. Roots and sprouts from other trees near the base of the tree in question, the appearance of branching from one of the tree's main roots which is actually due to the crossing over or under of a smaller root from another tree, and apparently also occasional natural grafting of roots may lead to collection of a sample from the wrong tree. Experience with these possible difficulties such as would be acquired by working with an Institute representative on the first samples, will make it clear what precautions must be taken. Then, for later collections, the reporting forester can make the collections with assurance, himself, and send in the root sample, for chromosome count.

Root samples can be taken at any time when the ground is not frozen, and should be kept moist in transit. Samples secured in June, July, and August produce sucker shoots very sparingly and if possible root collections should be made in the spring or fall. Portions of young leaves or root tips secured when the root cuttings are propagated in the greenhouse are killed in a fixative solution while in active cell division, stained, and then examined under the microscope, to determine the chromosome number.

During the winter--from December onward--short branches from the crown of the tree may be removed and placed in water in the greenhouse, to force development of leaf and flower buds. Especially favorable sample material from flower buds developing on these branches is available for counting the number of chromosomes, but the count may also be made from the young leaves which are formed from the leaf buds.

4. Follow-up

Trees which are confirmed as triploids will be reported back to the discoverer and then, together with him or his associate, given permanent marking (following a procedure eventually to be recommended as standard practice by a subcommittee of the Lake States Forest Tree Improvement Committee), reserved to spare them from cutting, completely described, and then devoted to whatever propagational and research activities are indicated.

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