A Morphological Analysis of Populus alba, P. grandidentata and their Natural Hybrids in Southeastern Michigan

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Summary

Populus alba L. was introduced from Europe to North America by early settlers and hybridizes naturally with the native American aspens, *Populus grandidentata* Michaux and *P. tremuloides* Michaux. Natural hybrids between *P. alba* and *P. grandidentata* are relatively common among spontaneously occurring tree hybrids in southeastern Michigan. This study was conducted to determine the morphological characters which best distinguish the taxa, and to determine if backcrossing and gene flow have occurred.

Eleven leaf, bud and shoot characters from 41 clones of the hybrid, 5 clones of *P. alba* and 22 clones of *P. granddidentata* were measured. Pollen abortion percentages of male hybrids were also determined. A hybrid index and a canonical variates plot were constructed using the morphological data.

Of the morphological characters measured, leaf blade width, number of teeth per margin, leaf pubescence and bud pubescence were found to best distinguish the parents and the hybrid. Pollen abortion percentages of the hybrids were higher than values for the parents and most of the hybrids had values which were higher than what could be expected for backcrosses. The hybrids were distributed somewhat closer to *P. grandidentata* than to *P. alba* in the multi-character analyses. This was not attributed to gene flow and it appears that backcrossing may not have yet occurred.

Key words: *Populus alba*, North American aspens, natural hybridization, morphological characters, natural variation, gene flow.

Zusammenfassung

Populus alba L. wurde durch die ersten Siedler von Europa nach Nordamerika eingeführt und bildete mit einheimischen amerikanischen Aspenarten wie *Populus grandidentata* Michaux und *Populus tremuloides* Michaux Hybriden. Natürliche Hybriden zwischen *Populus alba* und *P. grandidentata* sind unter den spontan auftretenden Hybriden im südöstlichen Michigan relativ häufig. Diese Studie wurde durchgeführt, um die besten morphologischen Merkmale zur taxonomischen Unterscheidung herauszufinden und zu bestimmen, wann Rückkreuzungen und Gene flow aufgetreten sind.

Elf Blatt-, Knospen- und Sproßmerkmale wurden bei 41 Hybridklonen, 5 Klonen von *P. alba* und 22 Klonen von *P. grandidentata* bonitiert. Der Prozentsatz abgestoßenen Pollens bei männlichen Hybriden wurde ebenfalls bestimmt. Anhand der morphologischen Daten wurden ein Hybridindex und eine Parzelle mit kanonischen Variablen kostruiert.

Von den untersuchten morphologischen Merkmalen waren Blattspreite, Anzahl der Zähne am Blattrand, Blatt- und Knospenbehaarung am besten zur Unterscheidung von Eltern und Hybriden geeignet. Der Prozentsatz abgestoßenen Pollens der Hybriden war höher als der der Eltern und die meisten der Hybriden hatten Werte, die wiederum höher waren als die bei den Rückkreuzungen erwarteten. Die Hybriden waren in der Multi-character-Analyse mehr der *P. grandidentata* als der *P. alba* zuzuordnen. Dies war aber nicht dem Gene flow zuzuschreiben und es schien, als ob Rückkreuzungen bis heute noch nicht zustande gekommen wären.

Introduction

Populus alba L., the European white poplar, was introduced to North America at least as early as 1785 (Cutler, 1785; Rehder, 1940). Though present in North America for probably at least 200 years, the tree has not yet become naturalized probably because planted individuals have been propagated from only a few clones, virtually all of which are female (Little *et al.*, 1957).

Hybridization between *P. alba* and the native aspens, *P. grandidentata* Michaux and *P. tremuloides* Michaux, has been reported in North America by many authors (Victorin, 1935; Little *et al.*, 1957; Barnes, 1961). The long occurrence of *P. alba* in North America and the seemingly widespread incidence of its hybrids suggests the possibility of backcrossing and eventual gene flow between *P. alba* and the native aspens. This phenomenon is most likely to take place between *P. alba* and *P. grandidentata* since most of the reported hybrids stem from these parents.

Little *et al.* (1957) have described the morphology of the hybrid with *P. grandidentata* (*P.* × *rouleauiana* Boivin), but no one has studied the natural populations to determine whether backcrossing is occurring. The objectives of this study were therefore to analyze the morphology of the hybrids of *P. alba* and *P. grandidentata* to determine what characters best distinguish them in the field and to look for evidence of backcrossing and gene flow.

Materials and Methods

During the spring and early summer of 1976, a search was made for hybrids of the native aspens, *P. grandidentata* and *P. tremuloides*, with *Populus alba*. Reconnaissance was conducted primarily from a car in parts of Washtenaw, Livingston, and Jackson counties in Michigan. Areas within these counties that contained undeveloped land or abandoned farms were carefully examined. The locations of *P. alba* trees were mapped and the areas surrounding these potential seed trees were searched on foot for hybrids.

The *P. alba* population in southeastern Michigan probably consists of a single clone since all the clones were female, flowered at the same time, and had standard deviations for leaf and shoot characters which were only about one half as great as those of the other taxa.

Leaf and twig collections were made from putative hybrids and parents during July 1976, using the standardized collection methods of BARNES (1969). Four shoots 2.5 to 7.5 cm long, bearing early leaves, were collected from the lower, inner crown of one tree in each clone. The leaves were pressed and dried, and the middle leaf from each shoot was used for measurements. Blade length, blade width, petiole length, number of teeth per side, midpoint (distance along the midvein from the widest part of the leaf to the base of the leaf blade), and shoot length were recorded. The ratios of blade width to blade length (BW/BL) and midpoint to blade length (MP/BL) were calculated for each measured leaf. In addition, the degree of shoot, bud, and leaf blade pubescence was scored for leaves and shoots using a relative scale from 0—6. This scale was expanded by a factor of ten for use in the numerical analyses.

The means and standard deviations of the above measurements were computed by clone and by taxa. A hybrid index, based on methods of ANDERSON (1948), was constructed using morphological data. In the hybrid index extreme characters of *P. grandidentata* received a hybrid index value of 0.0 and those characters of *P. alba* with the opposite extreme received values of 10.0. All other individuals of the parents and putative hybrids received values between 0.0 and 10.0 according to their positions relative to the extreme character states.

The morphological data were also analyzed using a canonical variates program of the University of Michigan Statistical Research Laboratory. In these analyses, the morphological data were transformed into canonical variates so that the differences among the taxa were maximized and the differences within each taxa were minimized. The first canonical variate accounts for the greatest variability between the taxa, and the second canonical variate accounts for the next greatest variability between the taxa. DANCIK and BARNES (1975) report that canonical variates analysis is well suited for demonstrating relationships among recognized taxa in order to study intermediateness and/or gene flow between parental populations.

Pollen was collected from cut branches of putative hybrids whose catkins had been allowed to mature in a greenhouse. *P. alba* pollen was obtained from a German source and from a male clone growing at the University of Michigan Matthaei Botanical Gardens. Samples of a least 1000 grains per clone were examined under a microscope, and the percentage of aborted grains was determined.

Table 1. — Means and F statistics of leaf and shoot characters of *Populus grandidentata*, *P.* × *rouleauiana* and *P. alba*.

Character	P. grandidentata	P. Xrouleauiana	P. alba	F[1] Statistic
Blade Length mm	86	77	58	18.2
Blade Width mm	78	64	48	30.1
Petiole L., mm	64	56	36	20.9
Tooth Number	9.2	8.1	5.5	23.2
Midpoint, mm	30	27	20	10.1
Shoot Length, mm	54	44	29	11.3
Leaf Pubescence	1	18	56	65.8
Bud Pubescence	38	52	56	59.9
Shoot Pubescence	20	37	51	15.4
BW/BL[2]	0.91	0.84	0.83	7.3
MP/BL[3]	0.35	0.35	0.36	0.06

[1]Differences among all character means (except BW/BL and MP/BL) of the three taxa are significant at $p<0.01$.
[2]Ratio of blade width to blade length
[3]Ratio of midpoint to blade length

Results and Discussion

A total of 41 putative natural hybrids between *P. alba* and *P. grandidentata* (*P.* × *rouleauiana*) were located in the study area (*Figure 1*). Only two putative hybrids between *P. alba* and *P. tremuloides* (*P.* × *heimburgeri* BOIVIN) were found, but because of their rarity they were not considered in this study.

Means of 11 morphological characters for 5 *P. alba* and 22 *P. grandidentata* clones and 29 clones of their putative hybrids are presented in *Table 1*. The mean of all the characters except MP/BL were approximately intermediate between the parents. The hybrids were more similar to *P. grandidentata* than to *P. alba* in the following characters: blade length, blade width, petiole length, tooth number, midpoint, shoot length, leaf pubescence, shoot pubescence. The hybrids were more similar to *P. alba* than to *P. grandidentata* only in bud pubescense and ratio of blade

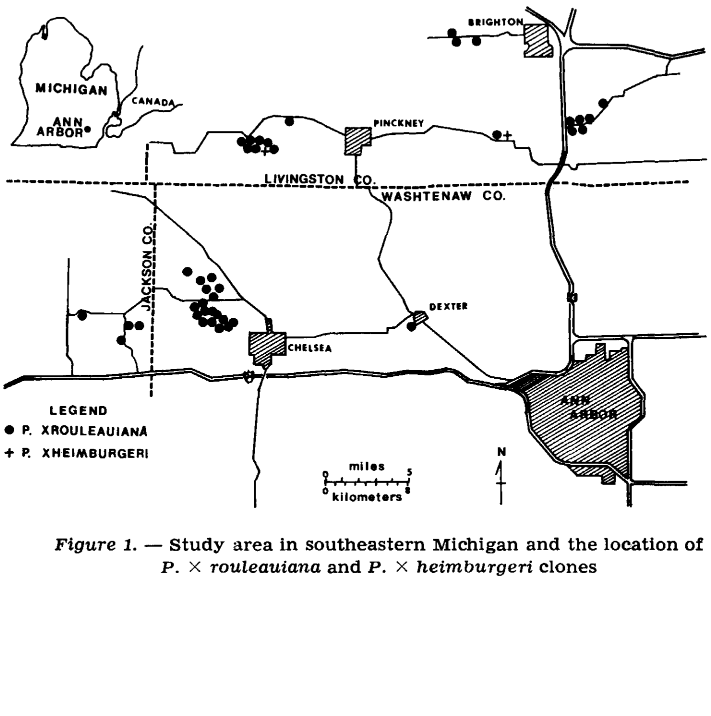

Figure 1. — Study area in southeastern Michigan and the location of *P.* × *rouleauiana* and *P.* × *heimburgeri* clones

width to blade length.

The results of analysis of variance indicated that the means of nearly all the characters (except BW/BL and MP/BL) of the three taxa were significantly different ($P < .01$) *(Table 1)*. Stepwise discriminant analysis was employed to help identify the characters most useful for separating the three taxa. Bud pubescence, leaf pubescence, blade width, and number of teeth per side were found to be the most important characters to discriminate the taxa. These were followed, in decreasing order, by blade length, midpoint, shoot pubescence, shoot length and petiole length.

Other field characters such as bark color and texture, tree form, and shape of late leaves were also helpful in distinguishing the taxa. *Table 2* presents a summary comparison of the taxa.

Pollen

The abortion percentages of 21 *P.* × *rouleauiana* clones averaged 15.3 and ranged from 8.5 to 25.5. The mean abortion percentages for two clones of *P. alba* was 6.5. No *P. grandidentata* was examined but BARNES (1978) found the mean abortion percentage of 21 clones in southern Michigan to be 3.9. He also reported a mean abortion percentage of 2.4 for 36 clones of *P. tremuloides*. PETO (1938) reported the mean abortion of 8 *P.* × *rouleauiana* and 3 *P. alba* clones to be 26.3% and 8.1%, respectively.

A high pollen abortion percentage is typically a good indicator of hybridity in the genus *Populus* (BARNES, 1978). The mean pollen abortion percentage found here for the putative hybrids is high enough to support their designation as hybrids.

Most of the putative hybrids had pollen abortion percentages that were higher than could be expected of backcrosses to *P. grandidentata*, because backcrosses in aspens typically have abortion percentages intermediate between the hybrid and the parents (BARNES, 1978). However, individual backcrosses may depart considerably from the midpoint between the parents. The expected pollen abortion

Table 2. — Comparison of *Populus grandidentata*, *P.* × *rouleauiana* and *P. alba*, using characters that best separate the taxa.

Character	P. grandidentata	P. Xrouleauiana	P. alba
Early leaf blade width (mm)	Mean = 78 Range = 55-101	Mean = 64 Range = 50-84	Mean = 48 Range = 43-54
Early leaf pubescence	Undersurface glabrous	Undersurface glabrous or often covered with patchy pubescence	Undersurface heavily tomentose
No. of teeth per margin of early leaf	Mean = 9.0 Range = 5-14	Mean = 8.2 Range = 4-12	Mean = 5.5 Range = 4-7
Lobation of late leaf	Unlobed	Shallowly three-lobed	Deeply three-lobed
Terminal bud pubescence	Lower scales pubescent, upper scales glabrous	Densely pubescent except scale margins visible	Heavily tomentose scale margins not clearly visible
Mature bark color	Tan, yellow or light green	Greenish or yellowish white	White, rarely yellowish white
Lenticels	Shallow, small, rhomboid shape	Rhomboid, deeper and more numerous than in P. grandidentata	Numerous, very deep, rhomboid
Branch angle	Spreading	Ascending	Acutely ascending
Lower branches	Not persistent	Less persistent than P. alba	Very persistent
Bole	Usually straight	Usually straight	Often crooked

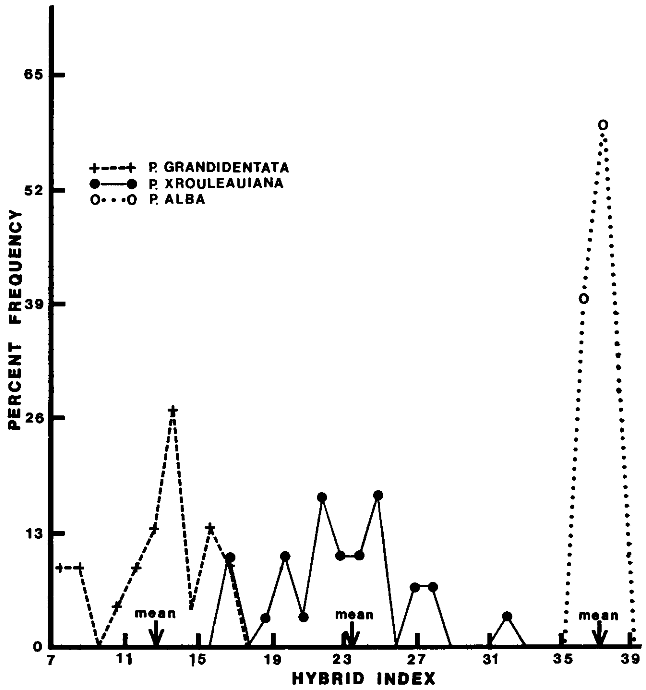

Figure 2. — Hybrid index of *P. alba*, *P. grandidentata* and their hybrid, *P.* × *rouleauiana*. Four leaf, bud, and shoot characters were used.

percentage for backcrosses to *P. grandidentata* would be about 9.6%. This value was based on the mean hybrid pollen abortion percentages of open pollinated clones in this study and the mean abortion percentages of *P. grandidentata* taken from BARNES (1978). Only three hybrid clones, with pollen abortion percentages of 3.5, 9.9, and 10.4, seemed to fall into the backcross range. However, their overall appearances were not more like *P. grandidentata* than the other F_1 hybrids.

Multi-character analysis

A hybrid index based on those characters that best separate the parents (leaf pubescence, bud pubescence, blade width and tooth number) was constructed *(Figure 2)*. The mean hybrid indices for *P. grandidentata*, *P.* × *rouleauiana*, and *P. alba* were respectively 12.9, 23.3, 37.5. The hybrid mean was located closer to the *P. grandidentata* mean than to the *P. alba* mean. There was some overlap between the hybrid and *P. grandidentata* distributions.

In the plot of the first two canonical variates the three taxa were well separated *(Figure 3)*. The hybrid was approximately midway between the parents, although somewhat closer to *P. grandidentata*.

The relatively wide distribution of the hybrid population in both the hybrid index and canonical variates analysis indicates that the population consists of many different genotypes. Phenological observations also support this interpretation.

The results of character measurements and these morphological analyses further reveals that the hybrids resemble *P. grandidentata* somewhat more than they do *P. alba*. However, this is not considered to be due to introgression or backcrossing.

The multi-character analyses *(Figures 2* and *3)* do not show an extensive intermixing of groups as would be expected if extensive backcrossing and gene flow had occurred. Rather, there was only slight overlap between the hybrid and *P. grandidentata* in the hybrid index and small deviation from the theoretical intermediate value of the hybrids in both multi-character analyses.

The overlap of the hybrid population and the *P. grandidentata* population in the hybrid index is due to three clones of the putative hybrids and two of *P. grandidentata*. Two of these hybrid clones, had pollen abortions of 13.9% and 19.4%, which are well above the expected backcross rate of 9.6%. These clones are probably variant F_1 hybrids rather than backcrosses.

Pollen abortion percentages were not available for the third hybrid, a female and the two *P. grandidentata* individuals, but the overall appearances of these individuals suggest that they are not backcrosses or introgressants.

In the canonical variates plot *(Figure 3)* two hybrid individuals and two *P. grandidentata* individuals were carefully examined since they were on the extremes of their group's distribution and proximal to the other group distribution. Hybrid clones 227 and 221 had pollen abortion percentages of 13.2 and 13.8. Based on these relatively high pollen abortions and their overall morphologies, these clones are considered F_1 hybrids. Pollen abortion percentages were not recorded for *P. grandidentata* clones 177 and 104. However, their overall characters do not resemble *P. alba;* they are most likely "pure" species.

The variant individuals described above were not the same in both multi-character analyses. This is probably because the hybrid index was based on four characters and the canonical variates analyses was based on nine characters. In spite of this, one might expect true backcross individuals to be clearly identified in both analyses.

The morphological variation of *P. grandidentata* in Michigan is quite wide (Barnes, 1969). *P. alba,* by contrast, is quite uniform, as evidenced by the narrow distribution of the *P. alba* population in *Figures 2* and *3*. The overlap in the hybrid index could be in part a result of the large morphological variability of the *P. grandidentata* population.

In addition the single-clone nature of the *P. alba* population makes the entire hybrid population half-sibs, possibly causing the hybrid population to deviate from expected hybrid index and canonical variates values. Farmer (1977) has found that family effects in *P. tremuloides* and in the hybrid of *P. grandidentata* and *P. tremuloides* were in part the cause of deviation from expected intermediacy as determined by canonical variates analyses. Thus the stronger resemblance of *P.* × *rouleauiana* to *P. grandidentata* may be due in part to the particular crossing results of a singular *P. alba* clone. The small sample sizes and relatively few characters used in this study, coupled with the peculiarities of the parental populations, are the most likely causes of deviation from hybrid intermediacy. If the *P. alba* population were natural and made up to many genotypes, a more truly intermediate hybrid population would be expected if the populations were sampled intensively.

The native aspens are so variable morphologically and the progeny from controlled crosses so variable (Farmer and Barnes, 1978) that morphological characters are difficult to use to distinguish conclusively backcrosses from F_1 hybrids. Farmer and Barnes (1978) reported that the large morphological variation in *P. tremuloides* in southeastern Michigan limits the reliability of morphological characters in detecting gene flow. They state that pollen abortion percentages and chemical techniques combined with morphological analyses may be more reliable methods for studying gene flow.

The evidence in this study from both morphological analyses and pollen abortion percentages does not indicate that widespread backcrossing and gene flow have ocurred in the direction of either *P. grandidentata* or *P. alba*. In fact, not a single clone could be interpreted as a backcross.

Acknowledgement

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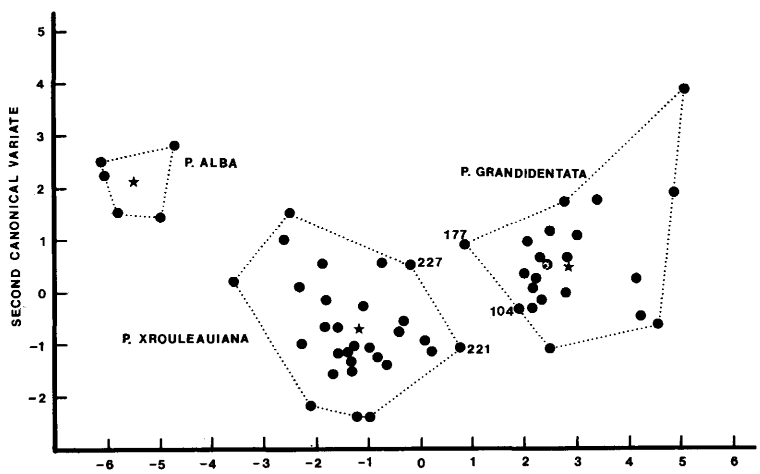

Figure 3. — Plot of canonical variates of leaf, bud and shoots of *P. alba*, *P. grandidentata* and their hybrid, *P.* × *rouleauiana*. Mean values of the respective taxa tre indicated by stars. Each point represents a single clone. Numbered clones are discussed in the text.

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Short Note: Index Selection with Restrictions in Tree Breeding

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Summary

Index selection is discussed for circumstances where restrictions can be imposed to either (1) limit changes in certain traits to zero, or (2) limit changes to a specific amount, or (3) maximise response in traits which for some reason are not included in the index. Mention is made of a computer program which will evaluate these restricted indices. Worked examples are given to illustrate their use in tree breeding.

Key words: Index selection, gain restrictions, computer program.

Zusammenfassung

Die Arbeit diskutiert die Index-Selektion, wobei folgende Einschränkungen gemacht werden können:

(1) Für bestimmte, der Selektion unterworfene Merkmale darf der genetische Gewinn (response) Null als Grenzwert nicht unterschreiten.

(2) Der Grenzwert wird auf einen bestimmten Betrag festgesetzt.

(3) Für Merkmale, die aus bestimmten Gründen nicht Bestandteil des Index sind, wird der Gewinn maximiert.

Es wird auf ein Rechenprogramm in FORTRAN IV hingewiesen, das diese eingeschränkten Indizes berechnet. Für einige Merkmale und zwei verschiedene ökonomische Gewichtungen ausgearbeitete Beispiele illustrieren die Anwendung in der Fortspflanzenzüchtung.

Introduction

Index selection was first described by SMITH (1936), and later HAZEL (1943), as a method of simultaneously selecting for many traits and is now widely used in tree breeding. Gains in total value form index selection are never less, but usually greater than, gains from comparable methods of selection (HAZEL and LUSH, 1942; YOUNG, 1961). However, in the long run index selection can sometimes lead to deterioration in individual traits. Under these and other circumstances restrictions may be imposed on the outcome of selection (JAMES, 1968). For instance, changes in certain traits may be restricted to zero (Kempthorne type restriction: KEMPTHORNE and NORDSKOG, 1959) or to a specific amount, perhaps to an optimum value determined by the market (Tallis type restriction: TALLIS, 1962), while maximum possible gains are made in the other traits. In another type of restricted index, gains can be maximised in traits which are of value but for some reason not included in the index (Binet type restriction: BINET, 1965; JAMES, 1968).

Index selection with restrictions is known to tree geneticists (NAMKOONG, 1979) but not commonly used. This note outlines a computer program which will evaluate selection indices with and without restrictions. Examples of index selection with restrictions are given for *Pinus radiata* D. DON in South Australia.

Computing

The junior author has written a FORTAN IV computer program which uses the algebraic methods of JAMES (1968), amended by MALLARD (1972), to evaluate both unrestricted and restricted indices in one extended computation scheme. The program is called RESI and a version which can combine up to 50 traits into one index occupies 29 k bytes of store (without overlay) on a 6 bit byte machine. A listing of RESI, a guide to its use, and test examples can be obtained from the senior author.

As input variables, RESI requires heritabilities, phenotypic variances and economic weightings for each trait to be combined in the index and genetic and phenotypic correlations between traits. Numerous sets of these parameters may be entered. Coefficients for index equations, expected responses in individual traits to selection on the index, and other variance and covariance information are determined for the unrestricted index, as well as any combination of Kempthorne, Tallis or Binet type restrictions.

An alternative method of computing restricted indices is given in CUNNINGHAM, MOEN and GJEDREM (1970).

Worked Examples

Unrestricted and restricted indices have been constructed by combining some or all of the following traits: diameter (underbark at 1.3 m) and volume, stem straightness and branch quality (measured as five-point visual scores: 1 = worst, and 5 = best straightness or best branching), and wood density (determined from torsiometer readings using the method of NICHOLLS and ROGET, 1977). Estimates of genetic and phenotypic parameters (*Tables 1* and *2)* used to determine these indices are from COTTERILL and ZED

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