A Study of *Populus* Species and Interspecific Hybrids Native to Southern Manitoba

by
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ABSTRACT

Four Populus species and numerous variants native to southern Manitoba were collected in 1967 and 1968. The range of variation and mean values for twenty-four morphological characters were obtained for P. tremuloides, P. balsamifera, P. deltoides var. occidentalis and three variant collections. The mean values for most characters in the variants collection were most nearly intermediate between the means of P. balsamifera and P. deltoides var. occidentalis. Phenological dates in 1968 revealed that a fairly effective seasonal isolation separates P. tremuloides from both P. balsamifera and P. deltoides var. occidentalis. The variants flowered at the same time as P. balsamifera and P. deltoides var. occidentalis. Most variants were found to be fertile and seed produced germinated nearly as well as in P. balsamifera and P. deltoides var. occidentalis. The fertility of the variants indicated that complex intragradation of the hybridizing species is possible. This is occurring in the Delta area where most introgression appears to be towards P. balsamifera. Eleven major compounds were observed in the chromatograms of methanolic bark extracts from the four species. It was possible to separate all four species on the basis of gas chromatography. The chromatograms of the variants were closely similar and had one major component that would appear to confirm P. balsamifera as their parent.

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INTRODUCTION

The genus Populus L. consists of a number of species mainly confined to the north temperate zone and of these, four have been listed as native to southern Manitoba (5, 20). P. balsamifera L. is characterized by lanceolate or orbicular-lanceolate leaves with whitish undersurface and low rounded serrations and by sticky reddish-brown buds with a balsam fragrance. P. tremuloides Michx. is characterized by round leaves with greenish undersurface and fine serrations and by shiny reddish-brown buds. P. deltoides Marsh. var. occidentalis Rydb. is characterized by deltoid leaves with green undersurface and coarse serrations and by yellowish buds. P. grandidentata Michx. is characterized by rounded leaves with greenish undersurface and coarsely toothed serrations and by greyish-brown, hairy buds. P. balsamifera, the balsam poplar, and P. tremuloides, the trembling aspen, occur throughout southern Manitoba while P. deltoides var. occidentalis, the plains cottonwood, and P. grandidentata, the large-toothed aspen are more restricted in distribution. Native P. deltoides var. occidentalis is mainly confined to the river valleys and lake shorelines of south-central and southwestern Manitoba while P. grandidentata is confined to southeastern Manitoba; thus at any one location only three species are found growing together in natural stands.

In the areas of Manitoba where the three species, P. tremuloides, P. balsamifera, and P. deltoides var. occidentalis are found a number of variants also exist. These variants are characterized by deltoid-lanceolate leaves with greenish-white undersurface, moderately coarse serrations and reddish-yellow buds. The variants are always found in the same area as P. deltoides var. occidentalis. These variants from Manitoba and elsewhere, including the cultivar 'Northwest', have been listed as hybrids by Scoggan (20), Boivin (4, 5), Brayshaw (9) and others (2).

The numerous reports of hybrid poplars, both in Manitoba and throughout Canada, have contributed to the difficulties of a taxonomic treatment of this genus. Flower characteristics have not been used to any extent in the preparation of identification keys. This can be attributed to at least three reasons; firstly the poplars are dioecious trees, secondly they do not flower regularly or when young, and thirdly the flowers are borne in catkins which usually develop and disintegrate before plant collections are made in the summer. Identification keys for the poplars have been based almost entirely on leaf morphology which has been shown to be highly variable (9, 11). It is impossible to identify most of the poplar variants on the basis of the identification keys available; in fact, in some cases hybrid species have been listed without any identification key being provided to identify them.

It has become increasingly evident that in order to identify poplars correctly, other characters must be studied and then incorporated into identification keys. The study of flower and biochemical characters could provide the taxonomist with new information to use in the identification of species and variants. If we are to use these characters we must know the range of variation for each character. The objectives of this study were to determine if a detailed morphological and chemotaxonomic study would delineate the species and resolve some of the taxonomic difficulties resulting from natural hybridization.

In past years poplar trees were often considered as weed trees that hindered the growth of conifers. However, recently the culture, breeding and taxonomy of poplars has been stimulated by their increased utilization in the lumber and paper industry, both in Canada and the United States. Native and introduced species and hybrids have also been important trees in the horticultural industry, especially in shelterbelt plantings.

LITERATURE REVIEW

The occurrence of poplars in Manitoba was noted by the early explorers and taxonomists.

In 1922, Jackson et al. (14), in a Check List of Manitoba Flora, listed P. balsamifera and P. tremuloides as native to Manitoba and noted that P. deltoides occurred as planted trees in Winnipeg.

P. grandidentata, P. balsamifera var. subcordata and P. deltoides were added to the list of native poplars by Scoggan (20) in 1957. He recorded a much wider distribution for P. deltoides and also noted the reports of hybrid poplars at St. Norbert and Glenboro.

In his Flora of the Prairie Provinces, Boivin (5) listed P. tremuloides, P. grandidentata, P. balsamifera var. balsamifera and P. deltoides var. occidentalis as native to Manitoba. He regarded the P. deltoides in Manitoba as being representative of the var. occidentalis or western form and he did not recognize the previously listed P. balsamifera var. subcordata as worthy of taxonomic rank. Boivin listed the occurrence in Manitoba of the following hybrids: P. deltoides var. occidentalis x P. tremuloides; P. balsamifera x P. tremuloides; P. balsamifera x P. deltoides var. occidentalis. He based his type for the hybrid of P. deltoides var. occidentalis x P. tremuloides on the 'Northwest' clone (4). This poplar has been considered (2) to be a hybrid of P. deltoides and P. balsamifera.

The four species native to Manitoba represent three of the sections of the genus Populus. Smith (21) listed P. tremuloides and P. grandidentata in the section Leuce, P. balsamifera in the section Tacamahaca, and P. deltoides in the section Ageiros.

Only one of the three areas of collections for this study has received previous detailed botanic study. The Delta marsh area was

studied by Löve and Löve (15) in 1954 and they recorded information on the structure of the ridge forest. They noted that aspen and maple dominated the middle zone of the ridge forest and that balsam poplar and cottonwood occurred in the middle zone. They made no reference to the occurrence of hybrid poplars along the forested ridge.

Although there are no reports of any previous detailed morphological study of poplar species and hybrids in Manitoba, a number of studies of hybridization between these same species have been reported from elsewhere. In a study of poplar in Michigan, Barnes (3) noted the common occurrence of hybrids of trembling aspen and large-toothed aspen. Although the two aspens reached their flowering peak at different times, Barnes found that some receptive flowers occurred on both species at the same time due to intraclonal variation. Intraclonal variation resulted from the delayed development of flowers at the base and tip of catkins. The intermediate hybrid nature of these aspen hybrids was shown by measurement of blade length and width, petiole length and leaf serration.

Brayshaw (9) studied poplars in Alberta in an attempt to resolve the parentage of hybrids he found growing along the river valleys. He used summer vegetative material and based his measurements on leaf form. He concluded that all the species were able to enter into the hybrid swarm with varying degrees of readiness. Trembling aspen was found to occasionally cross with cottonwood but not with any of the species in the section Tacamahaca. Many of the hybrids were thought to be a product of hybridization between three species.

P. trichocarpa and P. deltoides have been reported by Stettler (23) to hybridize freely under both natural and artificial conditions.

In a later study Brayshaw (10) studied, the two closely related species, P. balsamifera and P. trichocarpa and showed that the two species

had freely interbred over a large area of Alberta and British Columbia. P. balsamifera plants were characterized by having from twelve to twenty stamens, bicarpellate ovaries, and lanceolate, glabrous, two valved capsules, while P. trichocarpa plants had forty to sixty stamens, tricarpellate ovaries, and globose to subglobose, three valved, pubescent capsules. Plants from Saskatchewan and eastward showed P. balsamifera characteristics; those from western British Columbia showed P. trichocarpa characteristics while those from Alberta and eastern British Columbia were intermediate. He concluded that it was best to treat these two former taxa as sub-species of a single species. The name of P. balsamifera had priority according to nomenclatural rules, so was used for this new inclusive species.

Morphological studies carried out on poplars elsewhere have revealed a number of characters that might be useful in identifying Manitoba poplars. Nagaraj (18) studied cottonwood and trembling aspen in Illinois and found that the number of flowers in pistillate catkins varied from forty to sixty for cottonwood and from ninety to more than one hundred for trembling aspen. The seed number for each placenta varied from eight to fifteen for cottonwood and from six to seven for trembling aspen. The stamen number varied from forty to sixty for cottonwood and from five to fourteen for aspen. Stamen counts of sixty for P. deltoides and of eight or none for P. grandidentata have been also recorded by Fisher (13) in New York.

Maini (16) in a study of vegetative buds of trembling aspen, large-toothed aspen, and balsam poplar, showed that a distinct separation of the aspens and balsam poplars could be made on the basis of bud length.

Differences in flowering dates is an intrinsic, non-morphological feature that has been used in classification and construction of ident-

ification keys. Davis and Heywood (12) state that its value as a character in identifying plants increases with the degree of seasonal difference.

The dates of anthesis and seed shedding have not been used to any extent as an aid in the identification of interspecific poplar hybrids. Moss (17) recorded the dates of anthesis and seed shedding for trembling aspen and balsam poplar at Edmonton. He found that for trembling aspen the date of anthesis varied from April 11 to May 4, depending on the year, and that seed shedding occurred about six weeks later. Seed shedding dates for balsam poplar ranged from June 4 to July 2, depending on the year, while anthesis occurred forty to forty-seven days earlier. He also noted that the seed shedding date for western cottonwood usually occurred during the later part of July or early August.

Brayshaw (9), in his study of native poplars of Alberta, recorded the same flowering order for the species as had Moss. However, he did not record the flowering dates for any of the hybrids that he reported from Alberta.

The chemical constituents of plants and their relationship to a taxonomic classification has been studied by numerous workers. Alston and Turner (1) worked with Baptisia and showed that the species could be identified on the basis of compounds separated by paper chromatography. They showed that biochemical similarity paralleled morphological similarity and that hybrid combinations could be determined by the occurrence of compounds specific to the species involved. Bortitz (7,8) was able to identify a number of poplar species, mainly of European origin, by the paper chromatographic separation of their fluorescent compounds. He found that it was difficult to identify closely related hybrids. The age of the tree had no influence on the chromatograms.

The leaf oils of Picea have been used by von Rudloff (25,26,27) in chemotaxonomic studies. When analyzed by gas chromatography, white spruce and black spruce were found to have characteristic leaf oil distribution patterns. A hybrid of these two species was found to have a chemical composition similar to black spruce for five compounds, intermediate for five compounds and similar to white spruce for four compounds. This paralleled morphological results in which the hybrid was found to be similar to white spruce for 18 characters, intermediate for 23 characters and similar to black spruce for 7 characters.

The gas chromatography of the phenolic glycosides of Salix has recently been described by Bolan and Steele (6). Many of the phenolic glycosides listed for the genus Salix have also been listed as present in the closely related genus Populus. Steele et al. (21) have recently demonstrated the value of the technique in the screening of Salix species for phenolic glycosides. Their work with Salix has demonstrated the value of this technique in the study of quantitative variation of phenolic glycosides.

MATERIALS AND METHODS

The maps, keys and results presented are based on over 600 collections of Populus made throughout southern Manitoba, mostly south of the 51st parallel, in 1967 and 1968. Field collections in 1967 showed that a number of Populus variants were distributed throughout much of southern Manitoba and could usually be found wherever P. deltoides var. occidentalis occurred.

Three extensive areas of variation were found in 1967 and the variants in these areas were studied more extensively in 1968. The Oak Lake collection from southwestern Manitoba, the Delta collection from south-central Manitoba, and the Grand Beach collection from southeastern Manitoba form a 150 mile transect across southern Manitoba. Although both the typical P. balsamifera and the form similar to var. subcordata were collected, in this study they were treated as one unit. The numbers and sources of material studied in detail are recorded in Table 1.

Table 1. Source and Identification of Material

Collections	Numbering Key	Source
Populus	#101-#130	Grand Beach sand ridge along east shore of Lake Winnipeg
Populus	#201-#230	Oak Lake sandhills
Populus	#301-#330	Delta forested ridge along south shore of Lake Manitoba
P. tremuloides	#401-#432	Delta area south of marsh
P. balsamifera	#501-#524	Delta area south of marsh
P. deltoides var. occidentalis	#601-#632	Delta forested ridge along south shore of Lake Manitoba
P. Jackii 'Northwest'		Research Station, Canada Department of Agriculture, Morden
P. grandidentata		Rennie, Betula Lake

Collection Methods

Collections were made from trees bearing reproductive material. Each tree was labelled so that subsequent collections could be made from the same tree. Where clones were growing close together, collection of material twice from the same clone was avoided by noting such characteristics as tree form, bark color and sex. Collection of material from fast growing shoots at the base of the tree was avoided.

Collection of samples from the areas of variation were made at random over the whole area. The areas of variation sampled were as follows: 5 miles long by 100-300 yards wide at Delta, 2 miles long by 1/4 mile wide at Oak Lake, and 1 mile long by 100-200 yards wide at Grand Beach.

Three collections, consisting of winter twigs, mature catkins, and mature leaves, were made from female trees. Collections of winter twigs and mature leaves were made from male trees. Part of the collection was pressed and dried for the herbarium while part of each collection was used as fresh specimens for study and dissection. A loan of additional material was made from the Plant Research Institute, Canada Department of Agriculture, Ottawa (DAO). The herbaria of the former institute and of the University of Manitoba (WIN) were also examined in the course of this study.

Morphological Study

Winter Twigs

The characters studied on each sample were as follows: length and width of ten terminal vegetative buds on short growing laterals, the length and width of ten flower buds, number of flowers for three catkins, stamen number for three flowers, bud scale number for three flower buds,

and the number of tips for ten bracts. The stamen number was found to vary from a high number at the base of a catkin to a low number at the tip so all stamen counts were made on flowers from near the center of the catkin. The bracts of male and female catkins were similar so data from both types of catkins was grouped together.

Female Catkins

The characters studied were as follows: catkin length from peduncle base to tip of highest ovary for ten catkins, length, width, and pedicel length for ten capsules, length and width for ten seeds.

Leaves

Leaf measurements on three leaves of each sample were made as follows: leaf blade length (L), leaf width at widest point (W), distance from leaf blade base to widest point (b), petiole length (P) and serrations on one side of leaf. The leaf measurements made are shown in Figure 1. Photographs of leaf collections of Populus species and variants are shown in Figures 2-9.

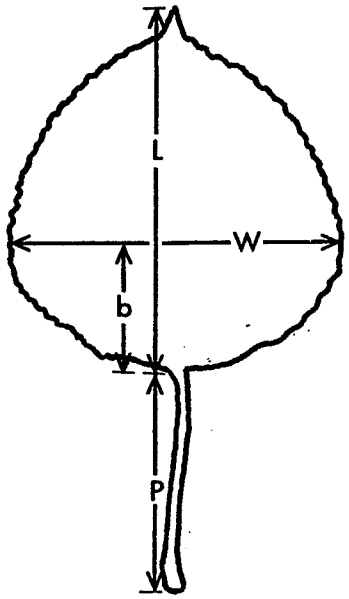
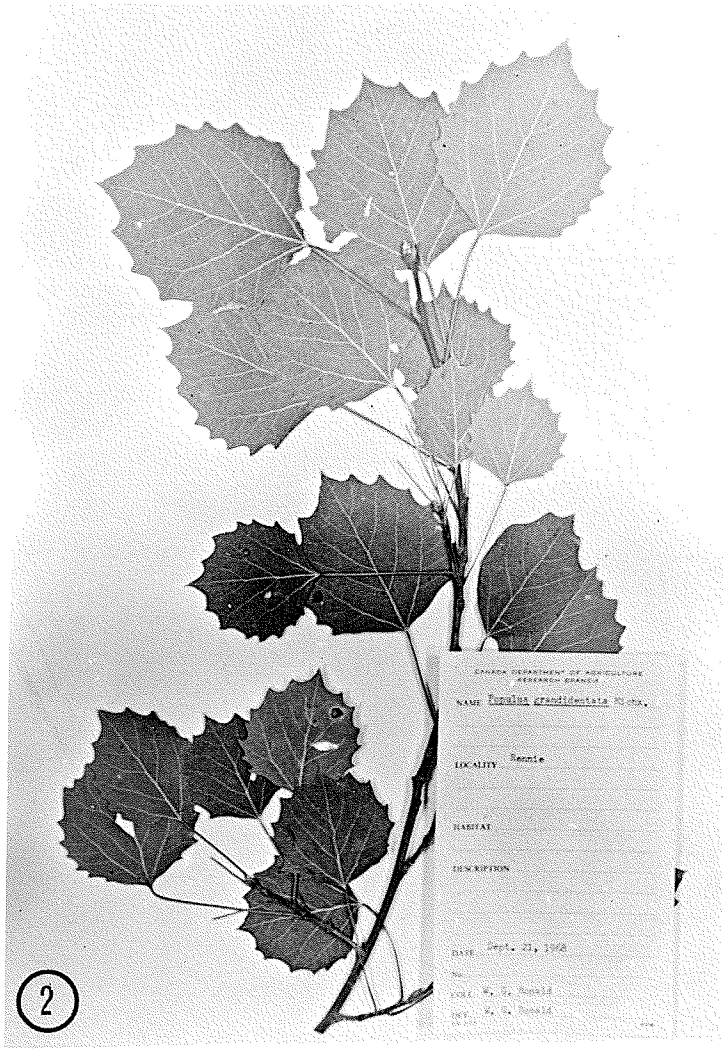


Figure 1. Measurements made on each leaf.



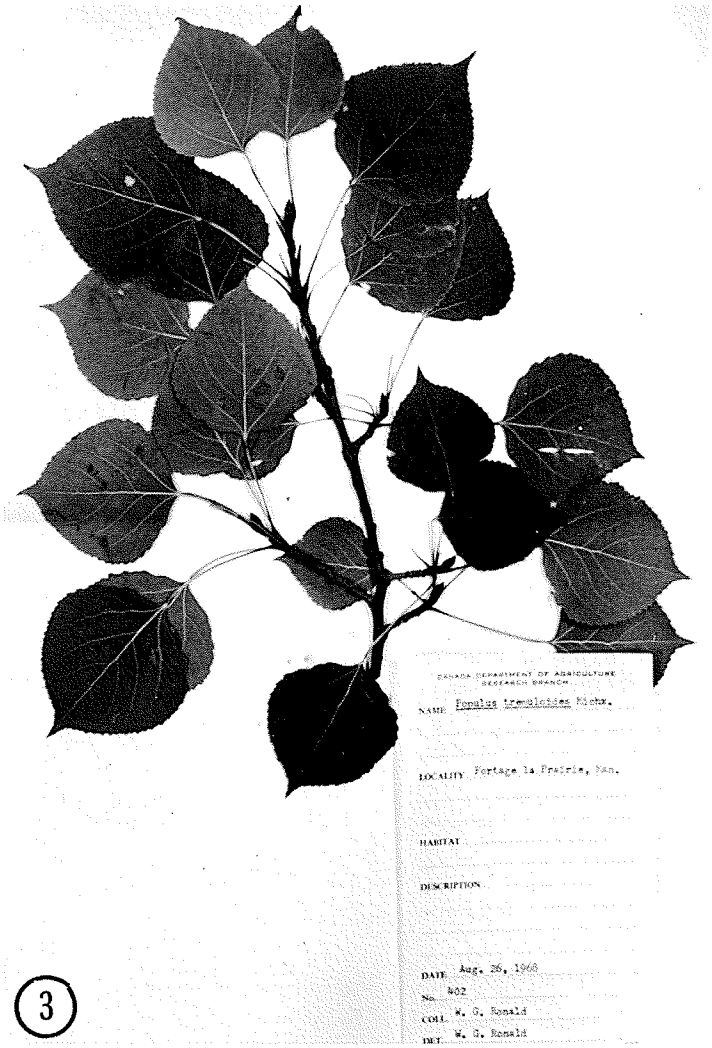
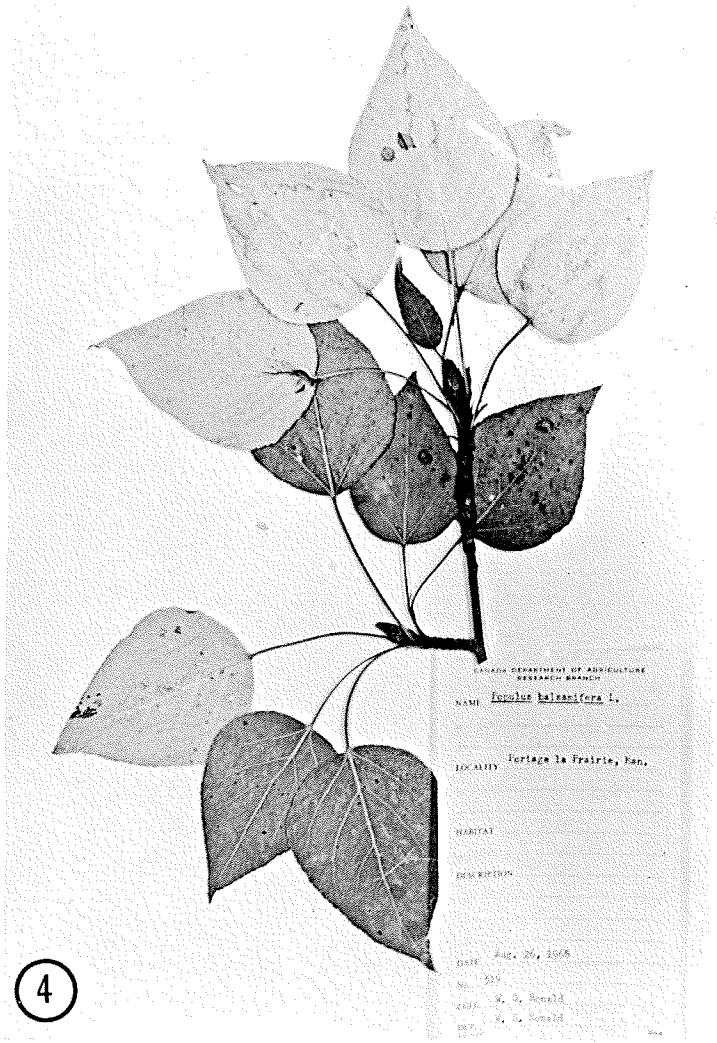


Figure 2. Specimen of P. grandidentata.
Figure 3. Specimen of P. tremuloides.



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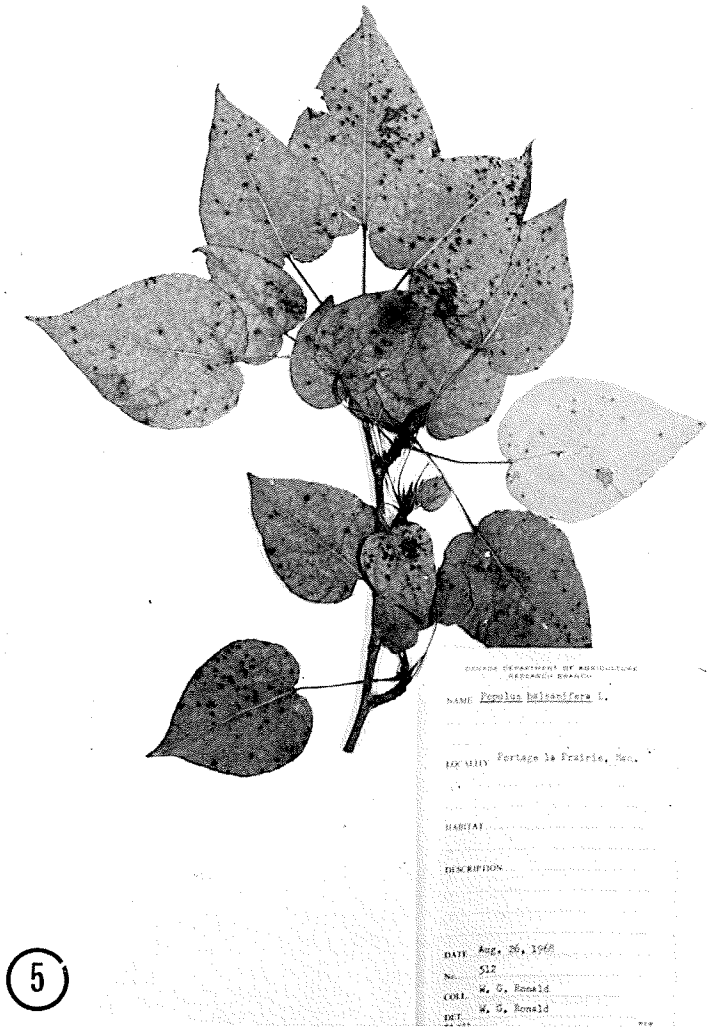

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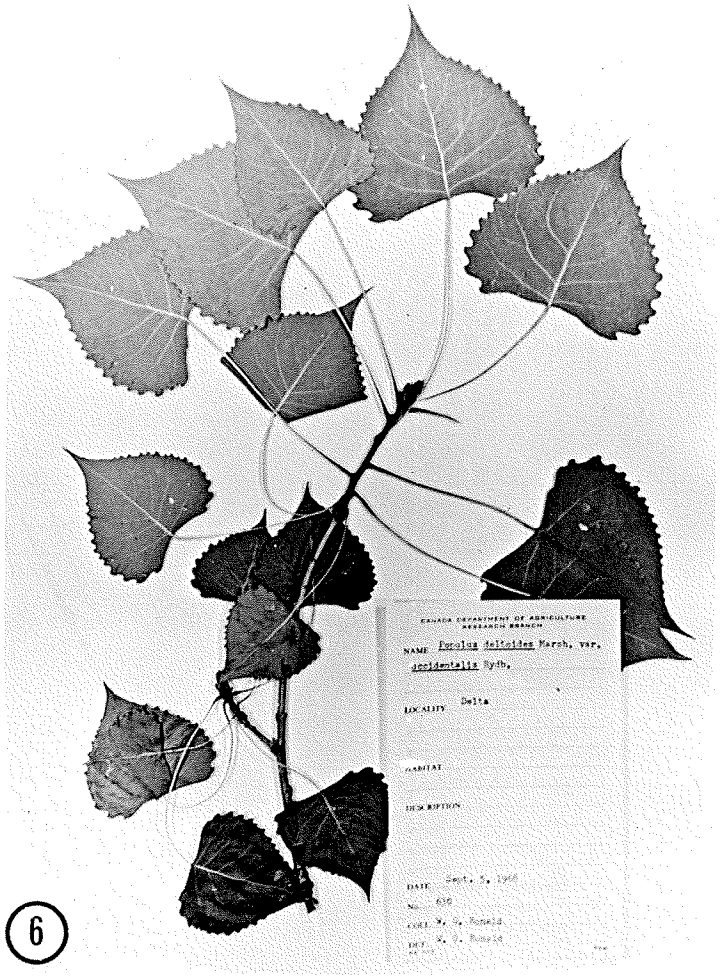
Figure 4. Specimen of P. balsamifera.
Figure 5. Specimen of P. balsamifera var. subcordata.



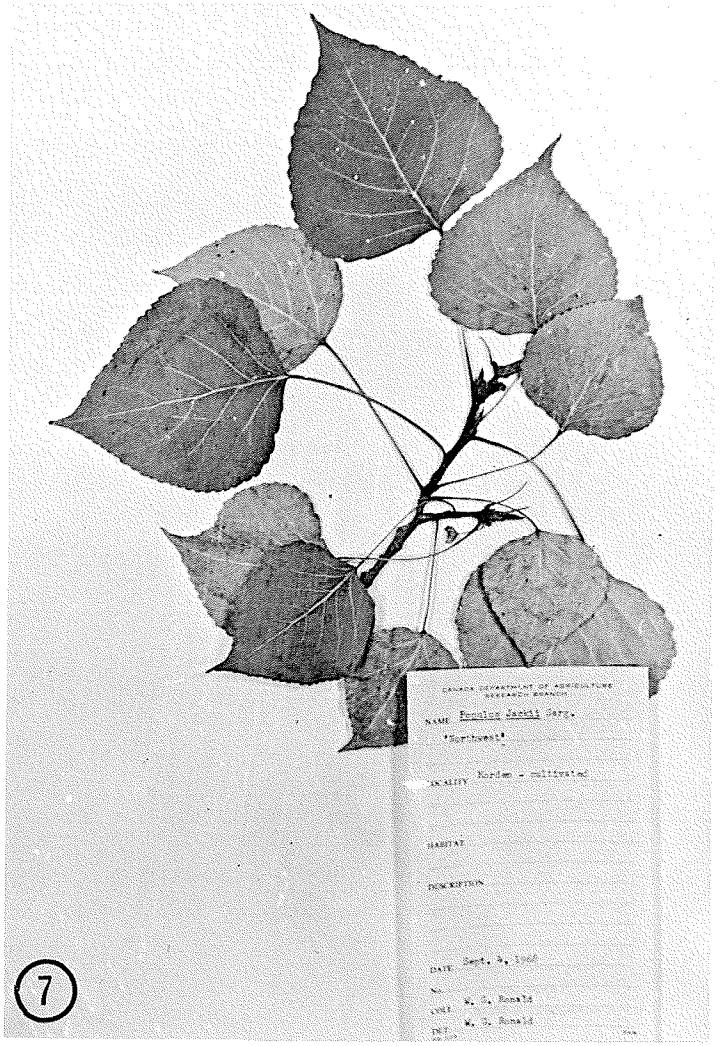


Figure 6. Specimen of P. deltoides var. occidentalis.
Figure 7. Specimen of P. Jackii 'Northwest'.

Figure 8. Specimen of Populus #316 resembling P. balsamifera.
Figure 9. Specimen of Populus #329 resembling P. deltoides var. occidentalis.

The following ratios were calculated from leaf measurements: distance from base to widest point of leaf/leaf blade length (b/L), leaf blade width/leaf blade length (W/L), and petiole length/leaf blade length (P/L).

The mean, of the measurements made on each numbered specimen, was calculated for each of the characters from winter twig, female catkin and leaf collections.

The range and standard deviation of the means, for each character, were calculated for the P. tremuloides, P. balsamifera, P. deltoides var. occidentalis and three variant collections.

Mean values for the 24 morphological characters were calculated, for each species and variant collection, by averaging the means from the individual numbered specimens.

Hybrid Index

A hybrid index based on 11 characters was drawn up for the 30 Delta variants and for the 'Northwest' cultivar.

Pictorialized Scatter Diagram

A scatter diagram, using the ratios of b/L and W/L as co-ordinates and based on 8 additional characters, was prepared for the Delta variants and 'Northwest' cultivar.

Phenological Study

The date of anthesis on male trees and of seed shedding on female trees was recorded for the species and variants in 1968. The date of anthesis was taken as the first day on which pollen began to be shed from catkins. The date of seed shedding was taken when the first seed capsules began to dehisce and seed became evident.

Fertility Study

The fertility of variants was compared with the fertility of two species by determining seed set and seed viability. The number of seeds in ten capsules was counted and this was expressed in terms of seeds per carpel.

The seed viability was tested by germinating 20 seeds on filter paper in each of two petri dishes. The seeds were germinated under a photoperiod of 12 hours light and 12 hours darkness. The temperature was held at 70°F for both light and dark periods. Filter papers were moistened each day with distilled water. Seed from six trees, of both P. balsamifera and P. deltoides var. occidentalis, was tested along with all variants that produced sufficient seed. Seed embryos that made any growth were considered to have germinated. Seedlings that were too weak to support their cotyledons above the filter paper were classified as weak seedlings.

Chemotaxonomic Study

Small samples of bark were saved from plant collections at the same time as herbarium samples of mature foliage were being collected. Bark samples were air dried at room temperature.

Extracts containing phenolic glycosides were prepared by refluxing 40 mg of bark in 40 ml of methanol for 2 hours. The methanol solution was then poured off into a small evaporating tube and evaporated to dryness with a flash evaporator.

Samples for injection were prepared by adding 50 ul of Tri-Sil to the dried samples remaining in the evaporating tube. The tubes were shaken and then allowed to stand for 30 minutes. Two samples of each

phenolic glycoside extract were chromatographed. The first injection consisted of 2 ul of solution and as soon as this was chromatographed a second sample of 2 ul of solution and a small sample of trimethylsilyl tremuloidin was chromatographed. Varying concentrations of trimethylsilyl tremuloidin were used. Retention times for the compounds were recorded relative to the trimethylsilyl tremuloidin.

Separations of the phenolic glycosides were made on an F and M model 5754 gas chromatograph using a single OV-1 column as prepared by Bolan and Steele (6). A hydrogen flame detector was used and the signal was recorded on a F and M linear potentiometer recorder set to a range of 1 mv. An attenuation of 2×10^2 and a chart speed of 0.25 inches per minute were used. Retention times were recorded with a Varian aerograph integrator. Helium, at a flow rate of 45 ml per min, was used as the carrier gas.

The following temperature program was used: inject at 185°C, hold for 4 minutes, then rise 4° per minute to 250° and hold until the last compound is eluted.

The usefulness of the prepared column, for the resolution of phenolic glycosides, was first tested by chromatographing trimethylsilyl derivatives of five reference compounds. These five reference compounds were as follows: salicin, arbutin, picein, tremuloidin and salireposide.

Bark extracts from a number of species and variant collections were chromatographed according to the procedure described above.

Collections of bark of P. tremuloides, P. balsamifera, P. deltoides var. occidentalis and the variants were made within a ten day period, from August 24 to September 3, 1968, so as to avoid a possible effect

of seasonal change in phenolic glycoside content. Collections of bark from P. grandidentata were made on September 21, 1968.

RESULTS AND DISCUSSION

Distribution of Species and Variants

The distribution of native P. grandidentata and P. deltoides var. occidentalis, based on collections and observations made in 1967 and 1968, is shown in Figure 10.

P. grandidentata is mainly confined to the extreme southeastern portion of the province that lies within the geological formation known as the Precambrian shield. Scoggan (20) listed P. grandidentata as occurring in southeastern Manitoba near Shoal Lake. In this study the most northerly location where this species was found growing was at Betula Lake, a distance of about 25 miles from the Ontario border and 60 miles from the United States border. This is about 25 miles northwest of the Shoal Lake collection reported by Scoggan.

P. deltoides and its forms have been widely planted throughout southern Manitoba and it is becoming increasingly difficult to distinguish native stands from stands made up of seedlings from introduced trees. The distribution as shown in Figure 10 attempts to show only those old mature stands that appear to be of natural origin. Native P. deltoides var. occidentalis occurs on disturbed sites and on sites having a periodically high water table. These conditions occur along the shoreline of rivers and lakes. The distribution of P. deltoides var. occidentalis was in agreement with Scoggan (20) who listed P. deltoides as occurring along the river valleys of southern Manitoba. The northward distribution of P. deltoides var. occidentalis would appear to be partly controlled by climatic conditions. The dominant position of this species, along the rivers, gives place to the boreal P. balsamifera in more northerly sections of Manitoba.

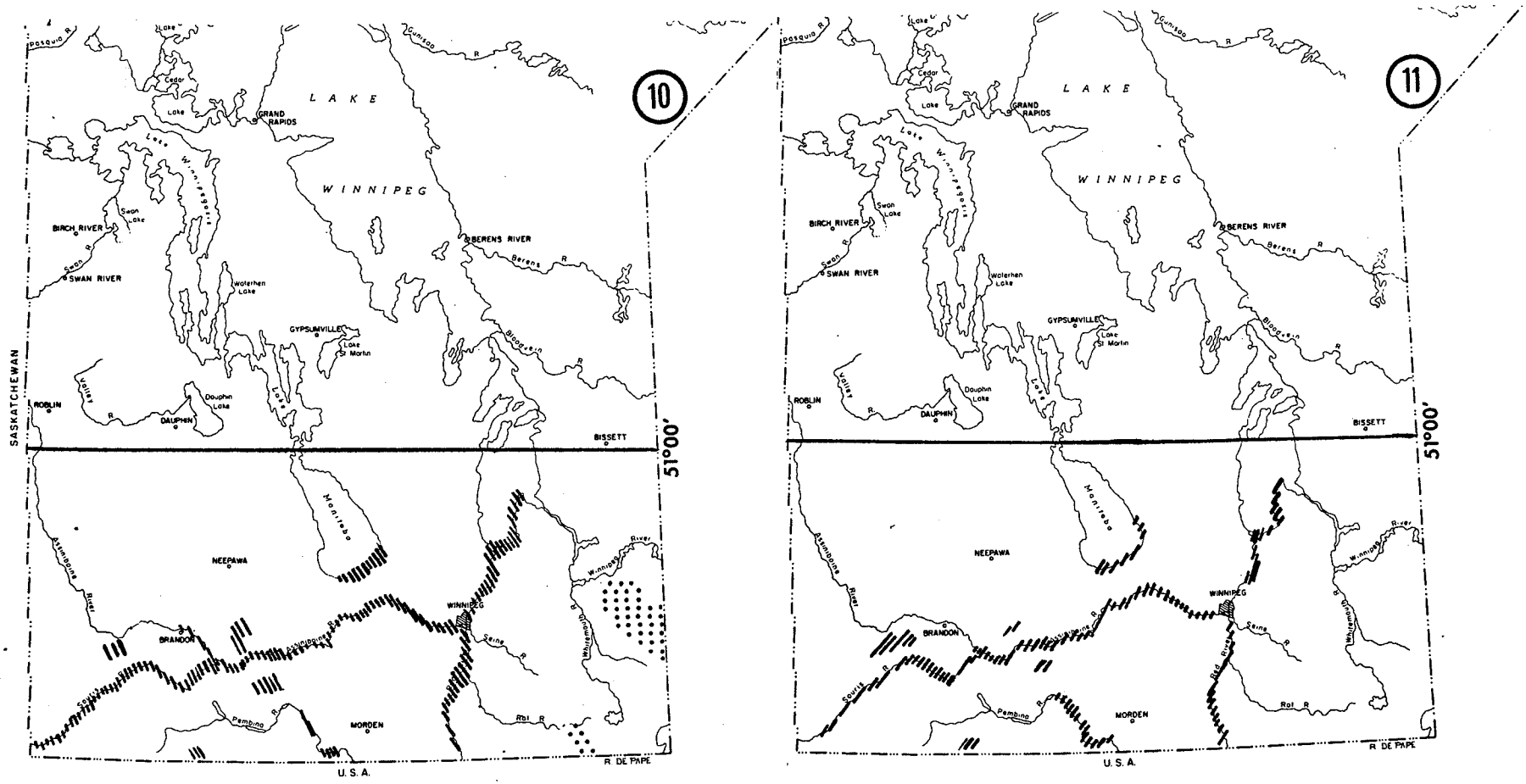


Figure 10. Distribution of P. grandidentata (dotted area) and P. deltoides var. occidentalis (hatched area) in southern Manitoba.

Figure 11. Distribution of naturally occurring poplar variants (hatched area) in southern Manitoba.

The distribution of natural variants, based on collections and observations made in 1967 and 1968, is shown in Figure 11. Natural variants are extensively distributed throughout southern Manitoba, and although they represent only a small percent of the total number of poplar trees, in many small localized areas they are more common than some of the species. In both the Oak Lake and Grand Beach areas, where variant collections were obtained, very few P. deltoides var. occidentalis trees were found. At Delta very few P. balsamifera trees were found along the forested ridge while variant trees were very common in this area.

The difficulty in the identification of variants is evidenced by the fact that they have often been confused with other species. Löve and Löve (15) appear to have identified the variants at Delta as either P. tremuloides or P. balsamifera. They mentioned that both P. tremuloides and P. balsamifera commonly occurred along the shoreline at Delta. Only a few clones of these two species were found in this area in 1967 and 1968 and it is believed that they identified the variants as either P. tremuloides or P. balsamifera.

Variants were not found in the area of Manitoba where P. grandidentata occurred, although hybrids between this species and P. tremuloides have been reported by Barnes (3) from Michigan.

Morphological Study

Photographs of a number of morphological structures are shown in Figures 12-28. Vegetative and flower buds are shown for P. tremuloides (Figure 12), P. balsamifera (Figure 13), P. deltoides var. occidentalis (Figure 14), and variants (Figure 15). Photographs of catkin bracts are shown for the four species (Figures 16-19) and for the cultivar 'North-

west' (Figures 20 and 21). Photographs of female catkins from five different trees are shown for P. tremuloides (Figure 22), P. balsamifera (Figure 23), P. deltoides var. occidentalis (Figure 24), and three variant collections (Figures 25, 26 and 27). The leaf shape and outline for a number of trees of both the species and variants are shown in Figure 28.

Mean values of 24 morphological characters for the Populus collections are shown in Table 2. The number of individual specimen means on which each aggregate mean is based, is listed beside the mean. The means for the characters in the cultivar 'Northwest' are based on one specimen.

The ranges, means and standard deviations for each character in P. tremuloides, P. balsamifera, P. deltoides var. occidentalis and three variant collections are shown in Figures 29, 30, 31, 32, 33, and 34. The horizontal line represents the range, the vertical line the mean, and the rectangle represents one standard deviation unit on either side of the mean. The means represented by the vertical lines in the center of the rectangle correspond to the means shown in Table 2.

Winter Twigs

All the means for the characters based on bud size were much greater for P. balsamifera and P. deltoides var. occidentalis than for either P. tremuloides or P. grandidentata (Table 2, Figures 29 and 30). The majority of the variant means were intermediate between, or slightly exceeded the means of P. balsamifera and P. deltoides var. occidentalis. Three of the variant means were slightly less than the comparable mean for either P. balsamifera or P. deltoides var. occidentalis, but in no case did the variant means approach that of P. tremuloides. As can be

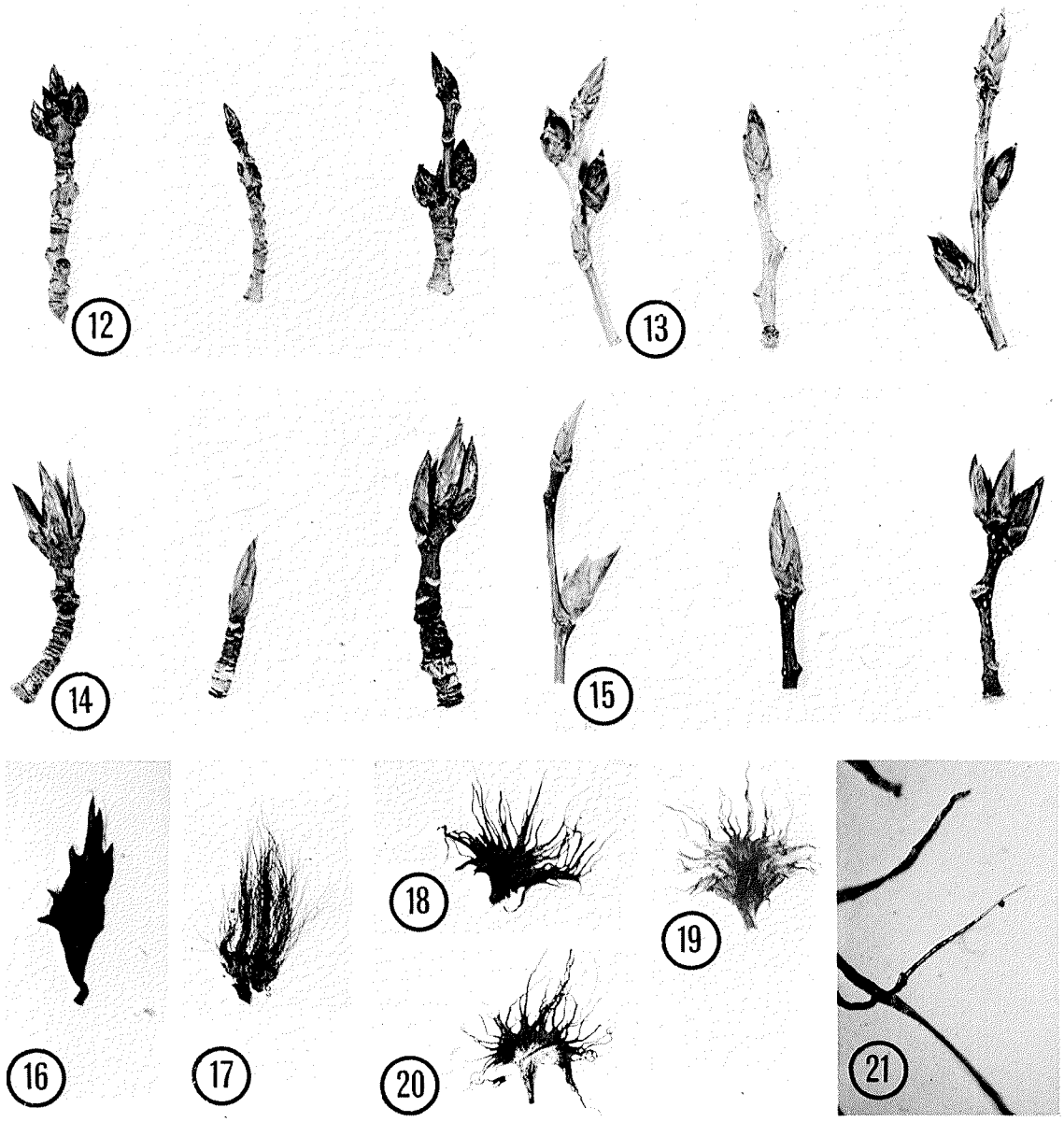


Figure 12. Buds of P. tremuloides showing male buds at left, vegetative buds in center, and female buds at right.
Figure 13. Buds of P. deltoides var. occidentalis showing male buds at left, vegetative buds in center and female buds at right.
Figure 14. Buds of P. balsamifera showing male buds at left, vegetative buds in center and female buds at right.
Figure 15. Male bud at left and vegetative bud in center of P. Jackii 'Northwest'. Female buds of Populus #310 at right.
Figure 16. Catkin bract of P. grandidentata.
Figure 17. Catkin bract of P. tremuloides.
Figure 18. Catkin bract of P. balsamifera.
Figure 19. Catkin bract of P. deltoides var. occidentalis.
Figure 20. Catkin bract of P. Jackii 'Northwest'.
Figure 21. Glandular type of hair on bract tip of P. Jackii 'Northwest' which is characteristic of P. balsamifera and most variants.

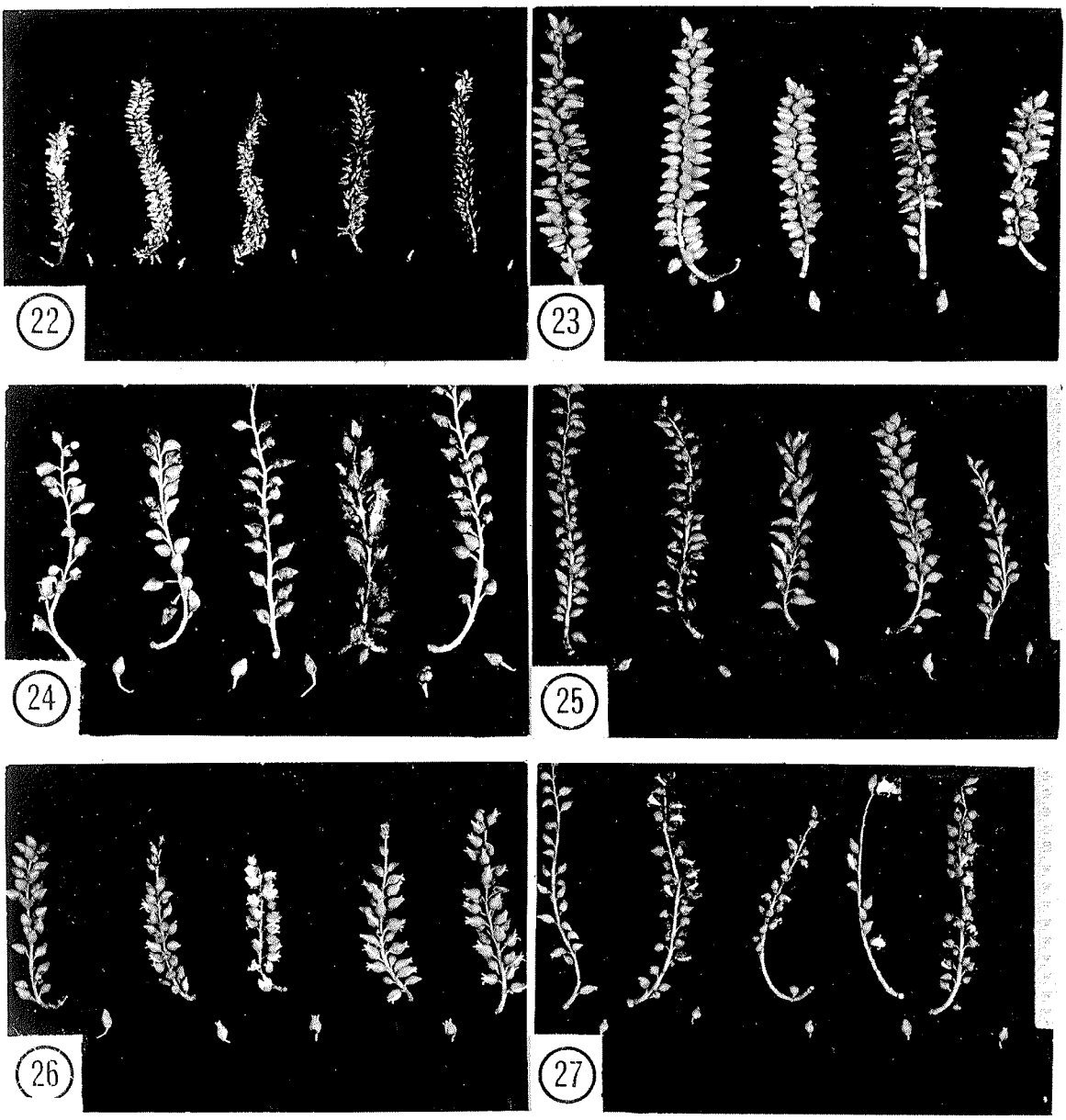


Figure 22. Female catkins of P. tremuloides.
Figure 23. Female catkins of P. balsamifera.
Figure 24. Female catkins of P. deltoides var. occidentalis.
Figure 25. Female catkins of Grand Beach variants.
Figure 26. Female catkins of Oak Lake variants.
Figure 27. Female catkins of Delta variants.

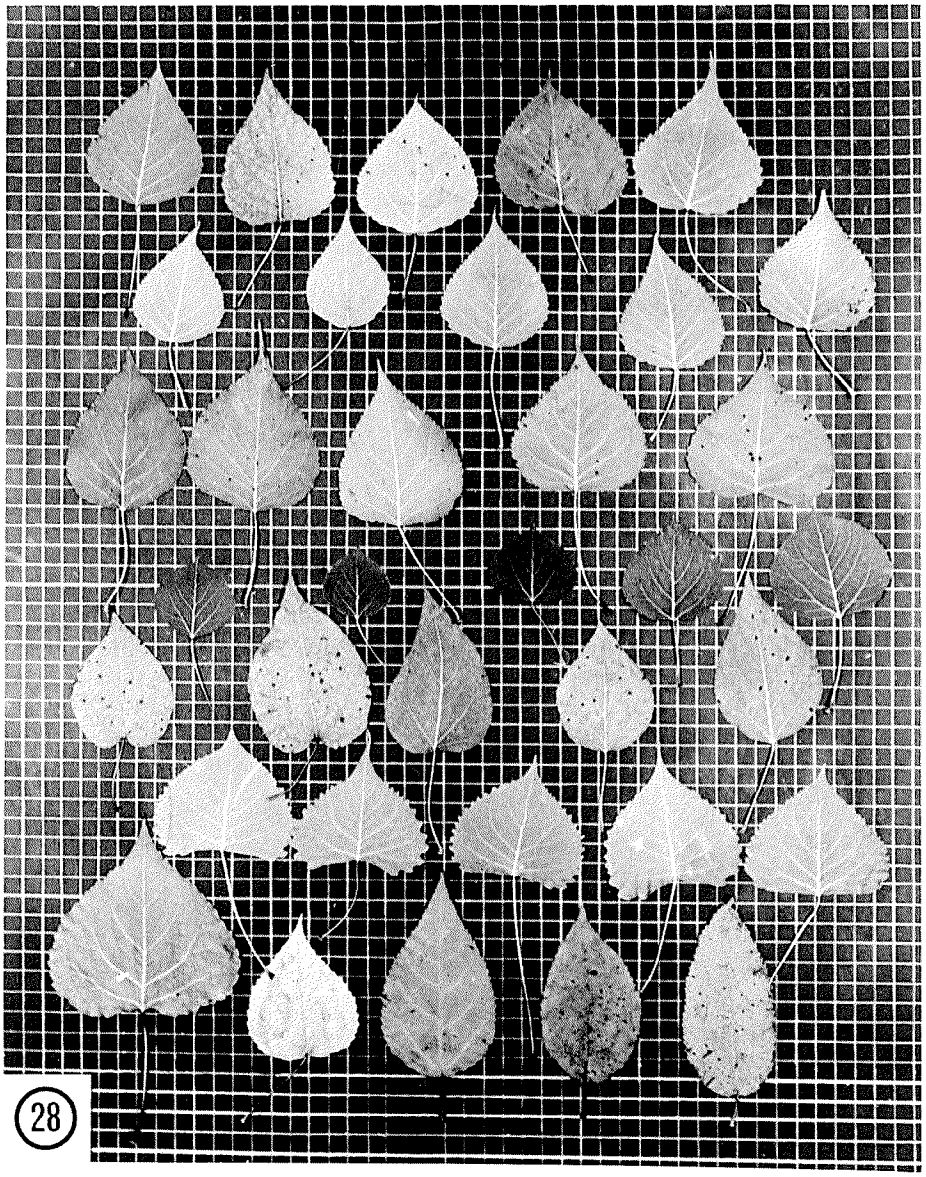


Figure 28. Leaves of poplar trees photographed against a square-centimeter grid. Leaves from Grand Beach variants (Row 1) and Oak Lake variants (Row 2). Leaves at left side of each row approach the shape of P. balsamifera while those at the right approach the shape of P. deltoides var. occidentalis. Row 3 shows leaves of Delta variants arranged in order of hybrid index (H.I.) from left to right as follows: #316 (H.I. = 5), #317 (H.I. = 11), #320 (H.I. - 14), #321 (H.I. - 15) and #329 (H.I. = 18). Row 4 shows leaves of P. tremuloides. Row 5 shows leaves of P. balsamifera with those of the left approaching var. subcordata and those on the right of the typical form. Row 6 shows leaves of P. deltoides var. occidentalis. Row 7 shows leaf of the cultivar 'Northwest' at left and a Populus variant from Holland second from left. Other leaves are from seedlings of the Populus variant from Holland.

Table 2. Mean values of 24 morphological characters in *P. grandidentata*, *P. tremuloides*, *P. balsamifera*, *P. deltoides* var. *occidentalis*, three variant collections and the cultivar 'Northwest'.

Characters	*P. grandidentata*		*P. tremuloides*		*P. balsamifera*		*P. deltoides* var. *occidentalis*		Delta Variants		Oak Lake Variants		Grand Beach Variants		'Northwest' Cultivar
	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	
Vegetative bud length (mm)	7.6	3	7.2	31	16.9	23	14.3	32	16.2	29	16.1	30	16.1	30	17.0
Vegetative bud width (mm)	3.7	3	2.6	31	4.0	23	4.2	32	4.4	29	4.2	30	3.9	30	3.9
Male flower bud length (mm)			10.5	12	17.9	10	17.1	17	18.6	11	18.0	9	20.3	23	18.8
Female flower bud length (mm)			10.0	9	17.6	7	14.5	15	18.1	18	15.9	21	16.9	7	
Male flower bud width (mm)			3.9	12	5.5	10	4.6	17	5.5	11	5.2	9	5.0	23	6.0
Female flower bud width (mm)			3.2	9	4.7	7	3.7	15	4.1	18	3.6	7	3.8	21	
Bract tips	5.3	3	6.4	21	27.7	24	55.5	32	55.6	29	58.1	30	55.5	30	42.6
Female flowers per catkin	120.6	3	108.8	16	58.3	12	33.1	15	51.7	18	39.9	21	51.2	7	
Male flowers per catkin			133.3	11	77.3	10	56.6	17	64.2	11	65.5	9	74.3	23	88.3
Stamen number			11.7	12	33.1	10	47.3	17	39.9	11	37.3	9	50.3	23	49.3
Female catkin length (cm)			9.9	16	11.3	12	13.1	10	14.2	13	10.4	19	13.0	7	
Capsule length (mm)			5.4	16	10.5	12	10.7	10	8.1	13	9.1	19	10.4	7	
Capsule width (mm)			2.3	16	6.0	12	6.5	10	5.1	13	5.5	19	5.1	7	
Pedicel length (mm)			1.6	16	1.7	12	4.1	10	2.4	13	2.6	19	3.0	7	
Seed length (mm)			1.4	16	2.0	12	3.5	9	2.8	11	2.6	19	2.6	7	
Seed width (mm)			0.6	16	0.9	12	1.3	9	1.0	11	1.0	19	0.9	7	
Teeth on one side of leaf	8.7	2	25.0	31	33.9	23	17.0	28	25.8	30	20.3	30	23.1	30	20.7
Leaf blade length (cm) (L)	7.1	2	5.4	31	9.3	23	7.6	28	8.7	30	6.6	30	7.8	30	8.6
Leaf blade width (cm) (W)	6.0	2	5.5	31	6.1	23	8.1	28	7.4	30	5.5	30	6.9	30	8.0
Petiole length (cm) (P)	5.3	2	4.3	31	5.1	23	7.6	28	5.8	30	4.4	30	5.7	30	6.9
Distance from base to widest point (cm) (b)	2.6	2	2.0	31	3.0	23	1.6	28	2.4	30	2.0	30	2.2	30	2.7
Position of greatest leaf blade width (b/L)	0.35	2	0.37	31	0.32	23	0.21	28	0.27	30	0.30	30	0.29	30	0.31
Leaf blade width/leaf blade length (W/L)	0.85	2	1.01	31	0.66	23	1.06	28	0.88	30	0.83	30	0.90	30	0.92
Petiole length/leaf blade length (P/L)	0.74	2	0.80	31	0.54	23	0.99	28	0.68	30	0.66	30	0.73	30	0.80

seen in Figures 29 and 30, most of the individual means for the variant collections fall within the range of the individual specimen means for P. balsamifera and P. deltoides var. occidentalis. In most cases, P. tremuloides and P. balsamifera showed less variation for these bud characters than P. deltoides var. occidentalis and the variants (Figures 29 and 30). The characters based on bud length served to reliably separate P. grandidentata and P. tremuloides from P. balsamifera and P. deltoides var. occidentalis. This is similar to the results of Maini (16) who found that a distinct separation of the aspens and balsam poplar could be made on the basis of bud length.

The mean values for the stamen number were quite different for each species (Table 2), although some overlap occurred between P. balsamifera and P. deltoides var. occidentalis (Figure 30). The means for the Delta and Oak Lake variant collections were most nearly intermediate between those of P. balsamifera and P. deltoides var. occidentalis, while the means for the Grand Beach variants and the cultivar 'Northwest' (Table 2) slightly exceeded the means of P. deltoides var. occidentalis. Both P. deltoides var. occidentalis and the Grand Beach variants showed a large variation in stamen number (Figure 30). The values obtained for stamen number in P. tremuloides and P. deltoides var. occidentalis were similar to those obtained by Nagaraj (18) for the same species in Illinois. Stamen number in P. balsamifera was found to be higher than that listed by Brayshaw (10) for P. balsamifera in our area. It would appear that stamen number may not be a useful character for the separation of the western and eastern varieties of P. balsamifera.

The number of tips on catkin bracts was low for P. grandidentata and P. tremuloides, high for P. deltoides var. occidentalis, and intermediate for P. balsamifera (Table 2 and Figure 30). The means for the

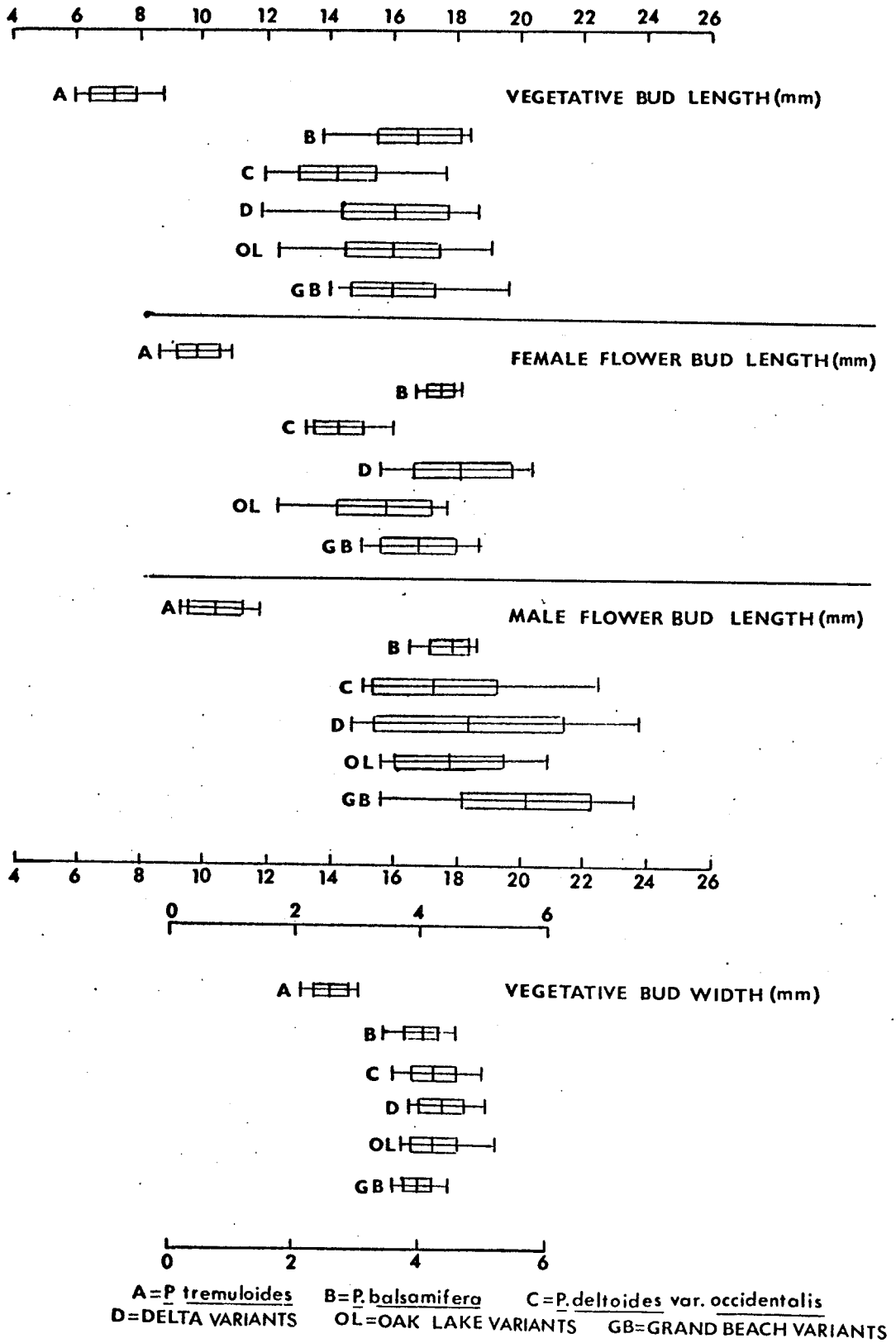


Figure 29. Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

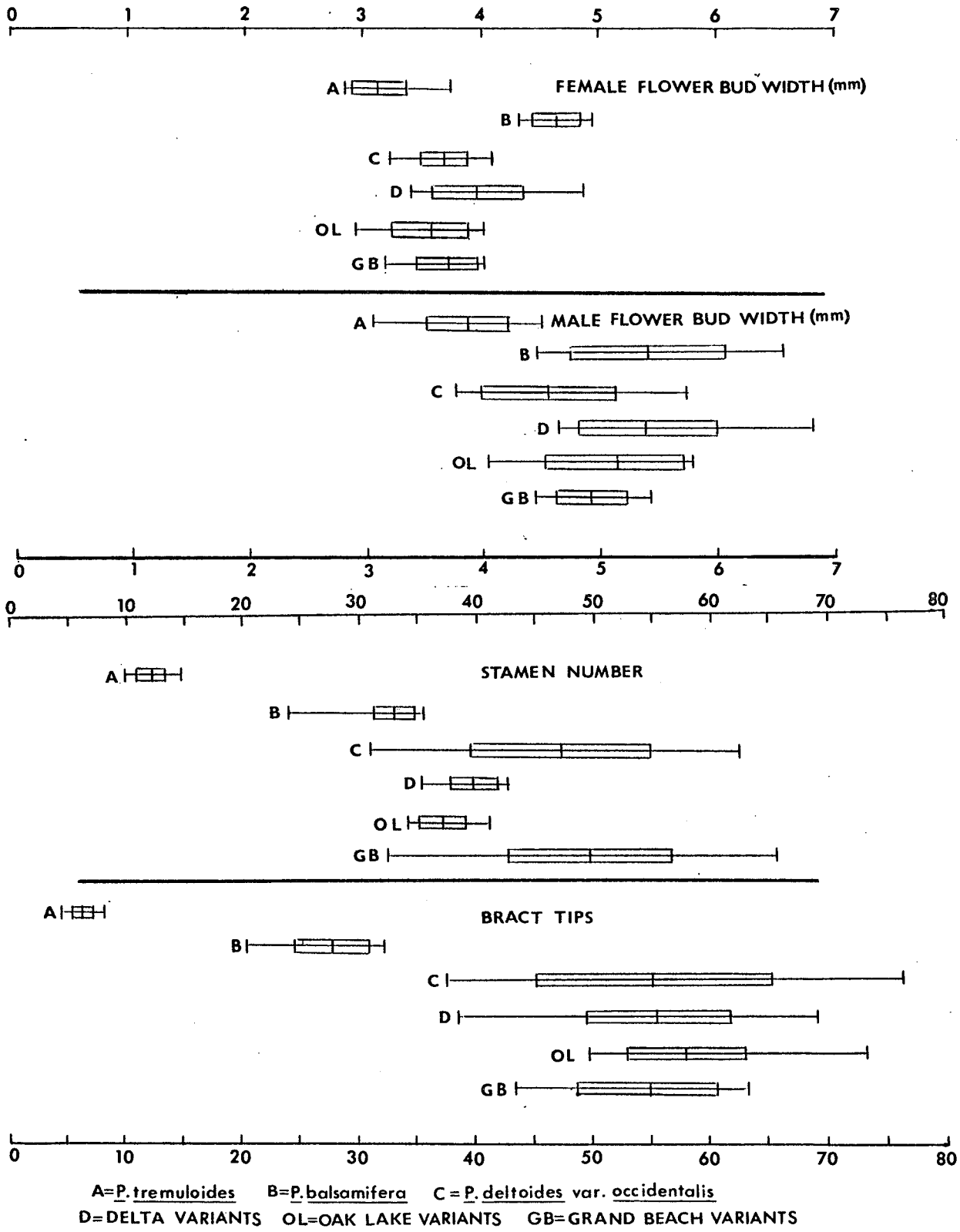


Figure 30. Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

three variant collections were similar to that of P. deltoides var. occidentalis while that of the cultivar 'Northwest' was most nearly intermediate between the means of P. balsamifera and P. deltoides var. occidentalis (Table 2).

The number of flowers in female catkins was much greater for P. grandidentata and P. tremuloides than for P. balsamifera and P. deltoides var. occidentalis (Table 2 and Figure 31). The mean values for the three variant collections were most nearly intermediate between the means of P. balsamifera and P. deltoides var. occidentalis (Table 2 and Figure 31). The number of flowers in female catkins for P. tremuloides and P. deltoides var. occidentalis were similar to numbers reported by Nagaraj (18) for these species in Illinois.

The number of flowers in male catkins showed the same trend as for female catkins with the mean values for the variant collections being intermediate between those of P. balsamifera and P. deltoides var. occidentalis (Table 2). The mean for the cultivar 'Northwest' was greater than the mean for either P. balsamifera or P. deltoides var. occidentalis (Table 2).

With the exception of male and female bud width, all ten characters measured on winter twig collections clearly separated P. tremuloides from both P. balsamifera and P. deltoides var. occidentalis. Most of these characters were of limited use in separating P. balsamifera from P. deltoides var. occidentalis. These characters were very useful for identifying variants as it was possible to tell whether the variant means were intermediate between P. balsamifera and P. deltoides var. occidentalis, or between P. tremuloides and P. deltoides var. occidentalis. The fact that the majority of the variant means were most nearly intermediate between those of P. balsamifera and P. deltoides

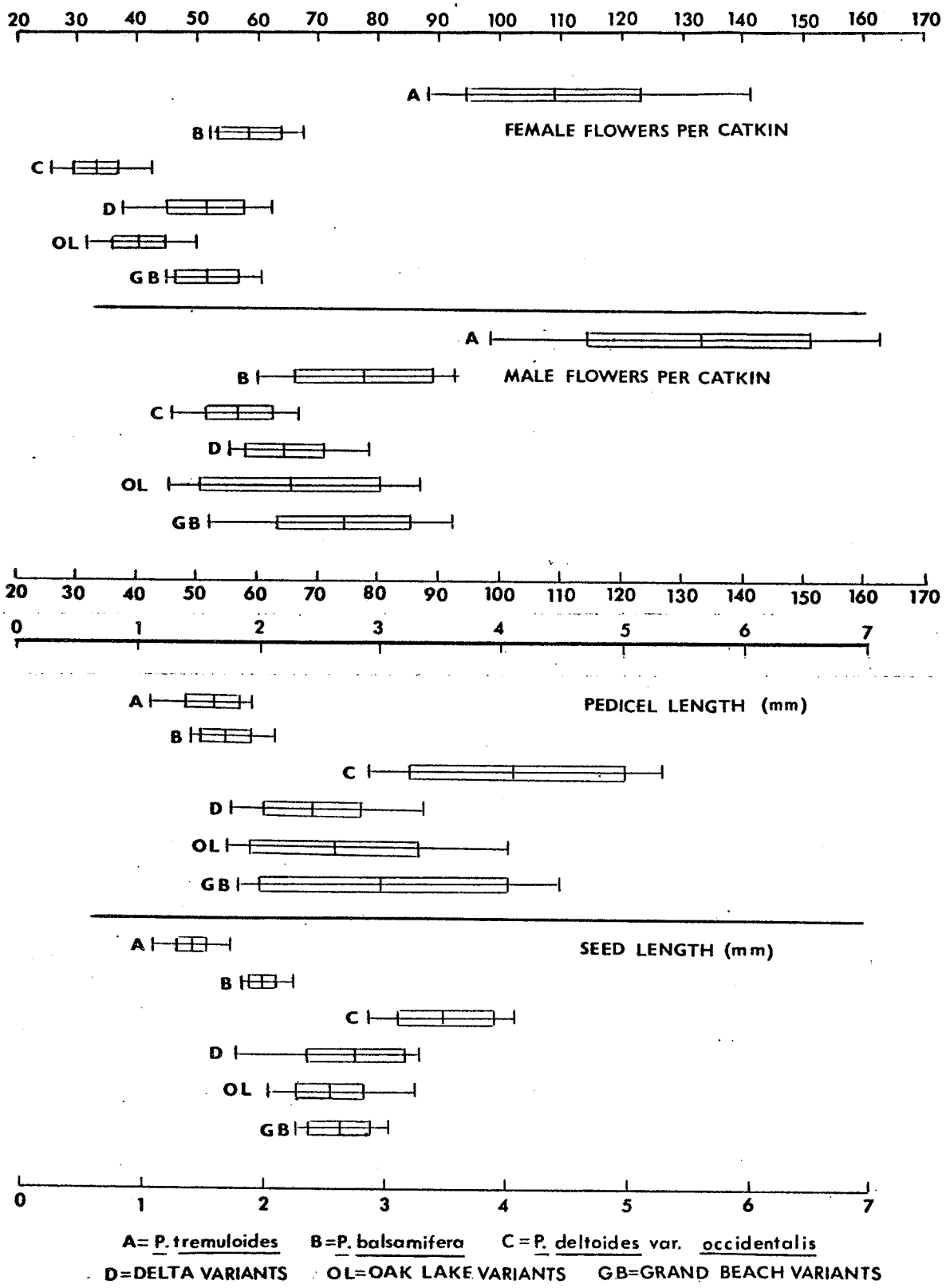


Figure 31. Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

var. occidentalis appears to indicate that these two species may be hybridizing.

Female Catkins

The pedicel length values shown in Table 2 and Figure 31 show that P. tremuloides and P. balsamifera are closely alike for this character, while P. deltoides var. occidentalis has much longer pedicels. Because the pedicel length values for P. tremuloides and P. balsamifera are closely alike, it is difficult to show that the variants are intermediate between P. tremuloides and P. deltoides var. occidentalis or between P. balsamifera and P. deltoides var. occidentalis. Pedicel length is a highly variable character in P. deltoides var. occidentalis and in the variants while P. tremuloides and P. balsamifera show less variation (Figure 30).

The mean values for seed length and width are different for the three species (Table 2, Figures 31 and 32). However, seed width in P. balsamifera and P. deltoides var. occidentalis shows some overlap (Figure 32). The values of seed length for the variants are most nearly intermediate between P. balsamifera and P. deltoides var. occidentalis, while the mean values for the seed width for the variant collections were equal to or slightly greater than that of P. balsamifera.

The mean catkin length values shown in Table 2 and Figure 32 are quite different for each species. However, this is a highly variable character as can be seen from Figure 32. The mean values for the variant collections are very different and collectively they do not fall into an intermediate position between any two of the species. Because of the wide variation in this character it would appear to be of very limited value in determining the identity of the species and variants.

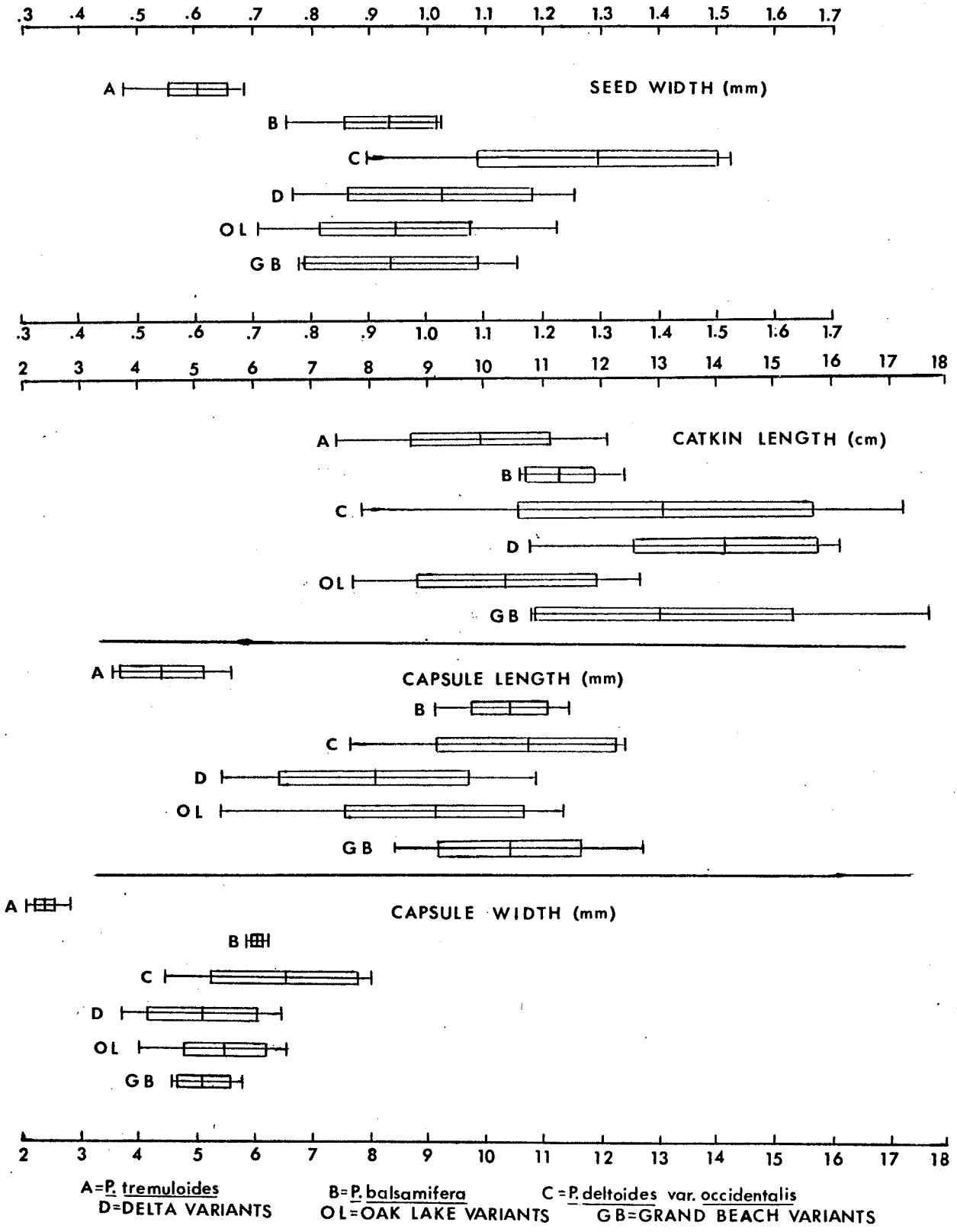


Figure 32. Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

The mean values for capsule length and width show that P. tremuloides is different from either P. balsamifera or P. deltoides var. occidentalis for these characters (Table 2 and Figure 32). The variation in P. deltoides var. occidentalis and the variant collections was larger than in either P. tremuloides or P. balsamifera (Figure 32). Values obtained for capsule length and width were always smaller for the variants than in either P. balsamifera or P. deltoides var. occidentalis. Since the fertility of the variants is usually very low, it would seem that full development of the capsule is dependent upon normal seed set. The variants with good seed set were found to have capsules intermediate in size between P. balsamifera and P. deltoides var. occidentalis. Variants with low seed set had small, misshapened capsules (Figure 27).

The six characters measured on female catkins would appear to be of limited value in determining the hybrid parentage of the variants. The mean values of seed width, seed length, pedicel length, and catkin length in the variant collections does indicate that P. deltoides var. occidentalis may be one of the hybrid parents. Due to infertility, many of the variants had poorly developed capsules and many aborted seeds. Thus the smaller values for these characters in the variant collections can be explained.

Leaves

The mean values for the number of teeth on one side of the leaf were different for each species (Table 2 and Figure 33). The means for the Grand Beach and Delta variant collections were most nearly intermediate between those of P. balsamifera and P. deltoides var. occidentalis. The values for the Oak Lake variants and the cultivar 'Northwest'

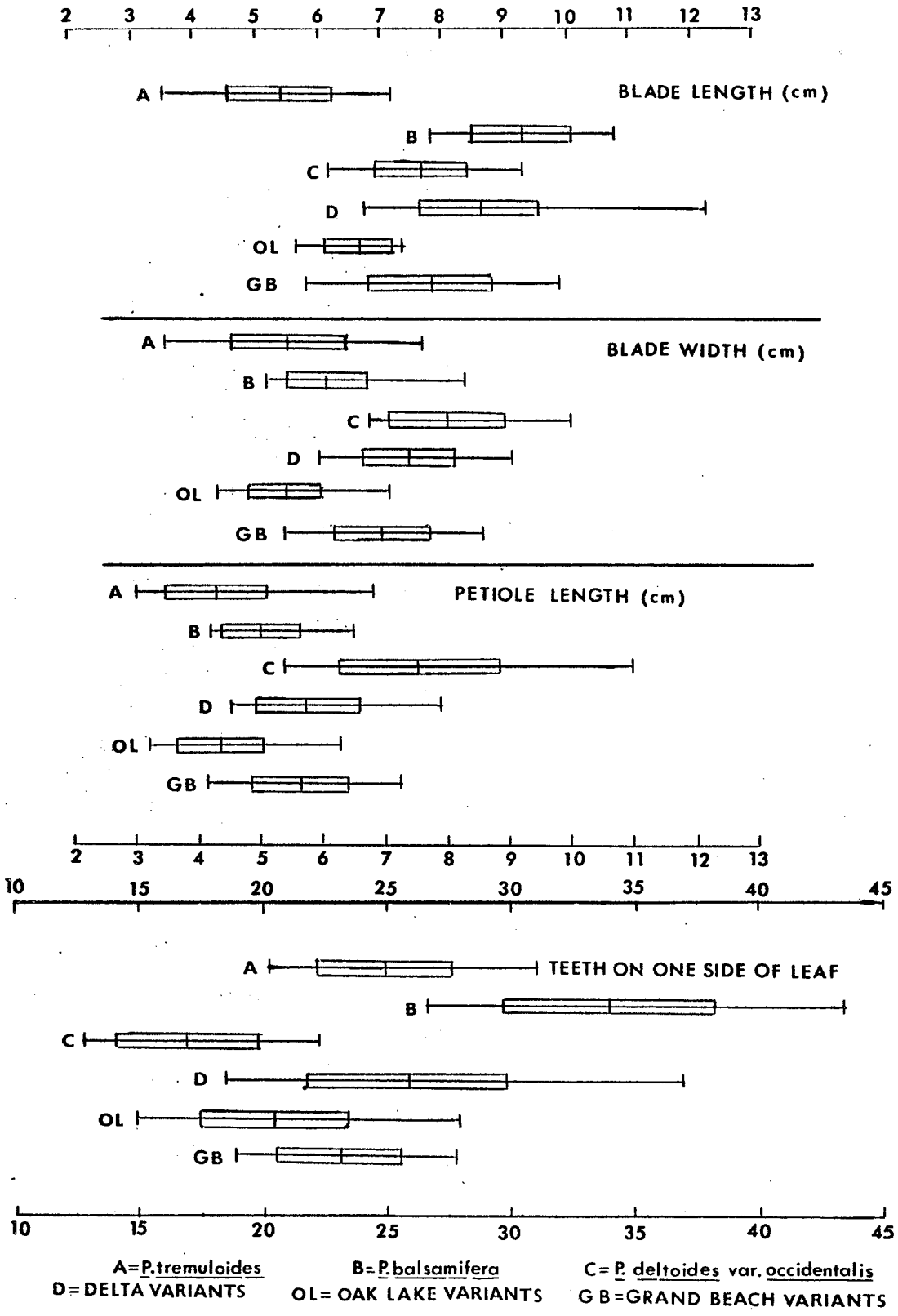


Figure 33: Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

were closer to that of P. deltoides var. occidentalis and thus approach a value most nearly intermediate between the means of P. tremuloides and P. deltoides var. occidentalis. This character is very variable in both the species and variants (Figure 33) and thus it has limited usefulness in identification of the species and variants.

The mean values for leaf blade length, leaf blade width, petiole length and distance from leaf base to widest point of leaf were quite different for the species collection (Table 2 and Figures 32 and 33). These characters were of limited use in separating the species as they were variable and species values overlapped. Most of the variant means, except those of the Oak Lake collection, were most nearly intermediate between P. balsamifera and P. deltoides var. occidentalis. The mean values for the Oak Lake collection were close to P. tremuloides (Table 2).

Mean values for the three characters based on leaf ratios are shown in Table 2 and the variation in these characters is shown in Figure 34. The variant values for the ratios of W/L and P/L (Table 2) appear to indicate that P. balsamifera is involved in hybridization as the means are much smaller than in P. tremuloides or P. deltoides var. occidentalis. The ratio of b/L (Table 2) does not appear to be as useful a character as the other characters based on leaf ratios. Results for this character were not consistent for the variant collections. The mean for the Delta collection was most nearly intermediate between the means of P. balsamifera and P. deltoides var. occidentalis, while the values for the Oak Lake, Grand Beach and 'Northwest' collections are most nearly intermediate between P. tremuloides and P. deltoides var. occidentalis.

It is interesting to note that mean values for leaf blade length, leaf blade width, petiole length, and distance from base to widest point of leaf were quite different for the Oak Lake collection as compared to

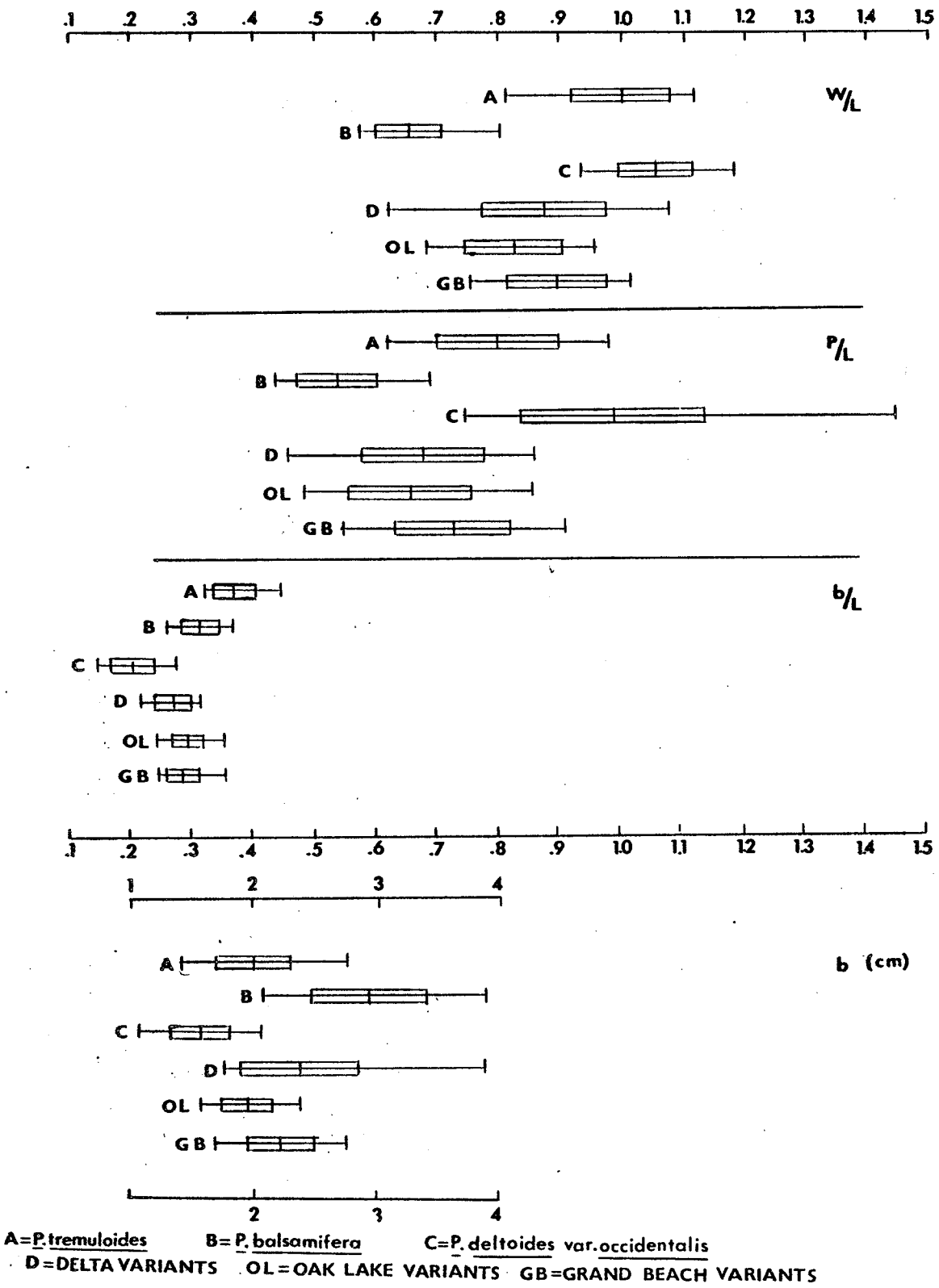


Figure 34. Ranges, means, and standard deviations of four morphological characters for three species and three variant collections of poplar.

the Delta and Grand Beach collections. However, the calculation of the leaf ratios W/L and P/L gives means that are nearly similar for the three collections. It would appear that the leaf size can be widely influenced by environment. The Oak Lake variants were growing under extreme drought and in very poor soil. The calculation of leaf size ratios appears to remove this environmental effect. The environmental effect found confirms results of Crovello (11) who reported that leaf characters were extremely plastic in the Salicaceae.

The Hybrid Index

The eleven characters used in this analysis and the value assigned to them are listed in Table 3. These eleven characters were used as they were common to both male and female trees and could easily be rated for each specimen. A plant characteristic of P. balsamifera should score 0, while a plant characteristic of P. deltoides var. occidentalis should score 28. All P. balsamifera scored by this method gave a hybrid index value in the range from 0-1, while all P. deltoides var. occidentalis scored in the range from 27-28. The hybrid index values for thirty Delta variants and the cultivar 'Northwest' were plotted against the frequency and the results are presented in Figure 35. The largest frequency of variants had hybrid index values of 15 or 16. There was a second peak at hybrid index value 12. A number of variants had values approaching those of P. balsamifera but only one variant value fell within the range of values for this species.

It appears that the majority of Delta variants are first generation hybrids as most of the hybrid index values are intermediate between those of P. balsamifera and P. deltoides var. occidentalis. A second peak of variants with hybrid index values of 12 may represent

Table 3. Hybrid index values of *P. balsamifera*, *P. deltoides* var. *occidentalis* and variants.

Character	Range	Index Number
Bud colour	Dark reddish-brown	0
	Light brown	1
	Tawny yellow	2
Colour of dorsal surface of leaf	Greyish-white	0
	Close to greyish-white	1
	Whitish-green	2
	Close to green	3
	Green	4
Leaf margin	Low rounded	0
	Crenate	1
	Crenate sinuate	2
	Moderately-coarse sinuate	3
	Very-coarse sinuate	4
Flower bud scales	4 or less	0
	Greater than 4 but less than 6	1
	6 or more	2
Hairs on bract tips of catkin	Abundant	0
	Frequent	1
	Sparse	2
	Absent	3
Hairs on upper surface of leaf at junction of petiole and blade	Abundant	0
	Frequent	1
	Sparse	2
	Absent	3
Petiole shape	Terete	0
	Slightly compressed	1
	Compressed	2
Leaf serrations	Greater than 27	0
	23 - 27	1
	Less than 23	2
Leaf blade width/leaf blade length (W/L)	Less than .81	0
	.81 - .93	1
	Greater than .93	2
Petiole length/leaf blade length (P/L)	Less than .65	0
	.65 - .75	1
	Greater than .75	2
Distance from leaf base to widest point (b) (cm)	Greater than 2.25	0
	2.00 - 2.25	1
	Less than 2.00	2

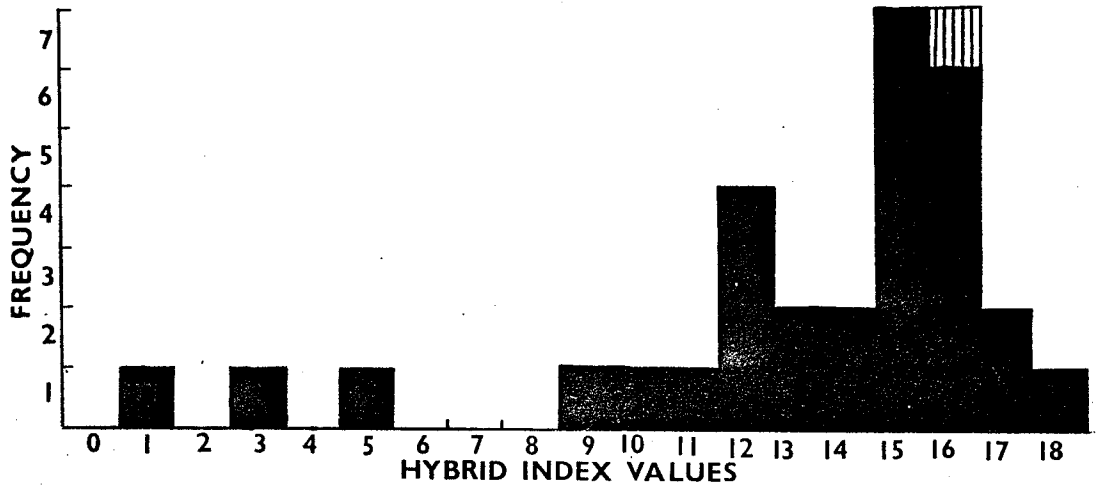


Figure 35. Frequency of hybrid index values of 30 Delta variants and the cultivar 'Northwest' (square with vertical lines).

progeny resulting from the backcross of F_1 progeny to P. balsamifera. Individuals with hybrid index values of less than 11, while showing a number of characters of P. deltoides var. occidentalis, were difficult to identify from P. balsamifera. It would appear that 'Northwest' is also an F_1 hybrid because it has a hybrid index value intermediate between those of P. balsamifera and P. deltoides var. occidentalis. There was little evidence of F_1 hybrids having backcrossed to P. deltoides var. occidentalis as no hybrid index values larger than 18 were recorded. Therefore introgression seems to be proceeding towards P. balsamifera.

A number of morphological characters including quantitative characters from the morphological study and qualitative characters from the hybrid index analyses were used to prepare summer and winter keys to the Populus. These keys are presented in the Appendix.

Figure 36. Explanation of symbols used in the pictorialized scatter diagram

Character	*P. balsamifera*	Intermediates			*P. deltoides* var. *occidentalis*
Petiole length/ leaf blade length	Less than 165		.65-.75		Greater than .75
	●		⊖		o
Petiole shape	Terete		Slightly compressed		Compressed
	o		-o		-o
Flower bud scale number	4 or less		Greater than 4 but less than 6		6 or more
	o		↘o		↘o
Glandular hairs on bract tips	Abundant		Frequent	Sparse	Absent
	o		o	o	o
Leaf serrations	Greater than 27		23-27		Less than 23
	o		o		o
Leaf margin	Low rounded	Crenate	Crenate sinuate	Moderately coarse sinuate	Very coarse sinuate
	o	o-	o-	o-	o-
Colour of dorsal surface of leaf	Greyish white	Close to greyish white	Whitish green	Close to green	Green
	o	o	o	o	o
Hairs on under-surface of leaf and petiole junction	Abundant		Frequent	Sparse	Absent
	o		♀	♀	♀

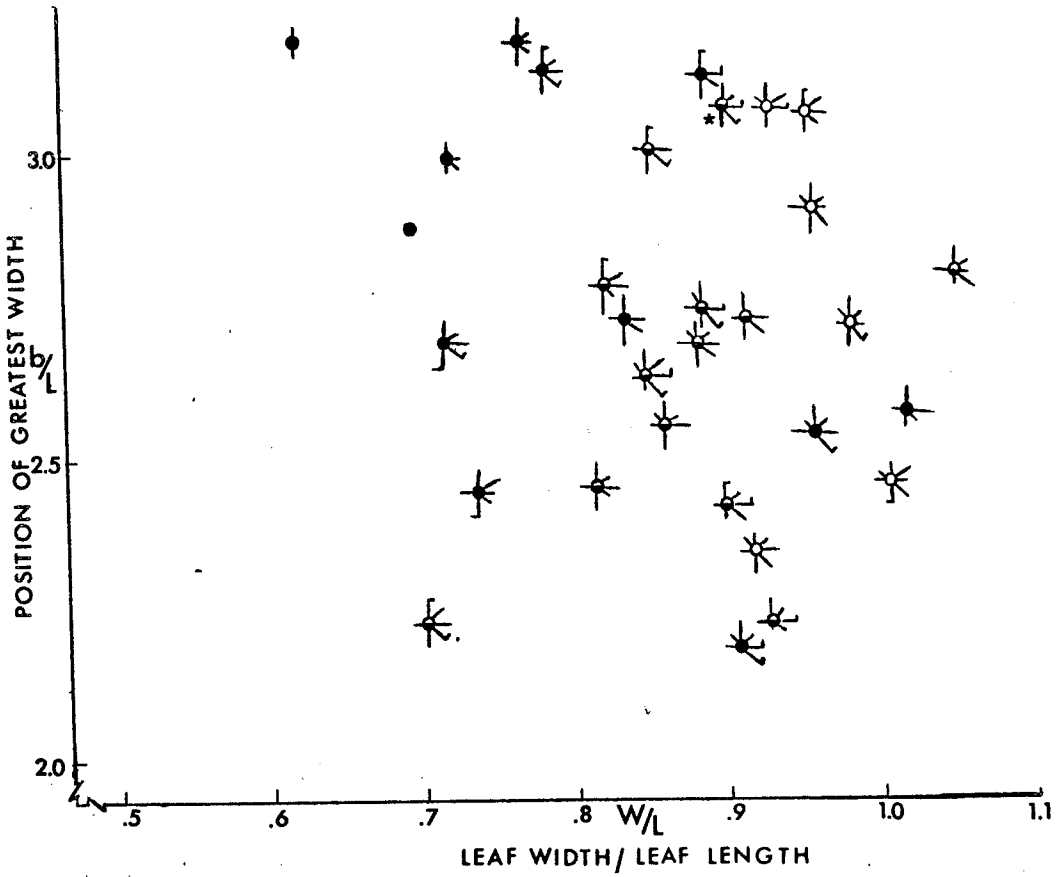


Figure 37. Pictorial scatter diagram of 30 Delta variants and the cultivar 'Northwest'. Diagram with asterisk = 'Northwest' cultivar.

Pictorialized Scatter Diagram

The eight characters used to modify the circle, formed by plotting each specimen against the co-ordinates, are shown in Figure 36. The pictorialized scatter diagram for the 30 Delta variants and the cultivar 'Northwest' is shown in Figure 37.

Evidence of the complex nature of the variants can be seen from the pictorialized scatter diagram. The majority of the variants were lumped together near the center. A few variants plotted in the upper-left hand corner showed mainly P. balsamifera characters, while individuals which were plotted in the lower-right hand corner showed mainly P. deltoides var. occidentalis characters.

Phenological Study

The dates of anthesis and of seed shedding are presented in Table 4. Marked differences, for both characters, exist between *P. tremuloides* and both *P. balsamifera* and *P. deltoides* var. *occidentalis*. As can be seen from the dates in Table 4, the variants are similar to *P. balsamifera* and *P. deltoides* var. *occidentalis*.

Table 4. Summary of phenological data for *P. tremuloides*, *P. balsamifera*, *P. deltoides* var. *occidentalis* and three variant collections.

Species	Location	Date of Anthesis	Date of Seed Shedding
P. tremuloides	Delta	Apr.18-Apr.22	May 22 - May 24
P. balsamifera	Delta	May 1 -May 4	June 19 - June 22
P. deltoides var. *occidentalis*	Delta	May 3 -May 8	June 19 - June 28
Populus	Grand Beach	May 4 -May 8	June 23 - June 30
Populus	Oak Lake	May 1 -May 3	June 20 - June 23
Populus	Delta	May 4 -May 8	June 25 - June 30

The dates of anthesis recorded in 1968 indicate that a fairly effective seasonal isolation exists between *P. tremuloides* and both *P. balsamifera* and *P. deltoides* var. *occidentalis*. This seasonal isolation explains the apparent lack of hybridization between *P. tremuloides* and either *P. balsamifera* or *P. deltoides* var. *occidentalis*. The overlapping flower dates for *P. balsamifera* and *P. deltoides* var. *occidentalis* may account for the apparent occurrence of hybridization between these species. Phenological data for the variants provides additional evidence to indicate that they are a result of hybridization between *P. balsamifera* and *P. deltoides* var. *occidentalis*.

The slightly later date of anthesis and seed shedding for the Grand Beach and Delta variants and P. deltoides var. occidentalis from Delta can be explained by the effect of the cold winds and air moving off the frozen lakes. This cold air movement may have held back the development of both anthesis and seed maturation in the early spring.

It has been suggested by Brayshaw (9) that once hybridization between the poplar species has been initiated, the hybrids could be expected to be intermediate in their flowering periods and thus form a phenological "bridge" between the parents. The results from our study indicate that both P. balsamifera and P. deltoides var. occidentalis have nearly similar flowering dates. The apparent hybridization of these two species does not appear to lead to a breakdown in phenological dates between them and any other species.

The phenological data recorded agree fairly closely with that found by Moss (17). The shorter time lapse, between anthesis and seed shedding, than that found by Moss may have resulted from climatic differences. Manitoba experienced very high temperatures in late April and early May of 1968, and this may have hastened seed shedding in P. tremuloides. The difference in the dates of seed shedding for P. balsamifera and P. deltoides var. occidentalis recorded by Moss in Alberta, did not occur in Manitoba.

Fertility Study

The fertility of P. balsamifera, P. deltoides var. occidentalis, and three variant collections, as measured by seed set, is shown in Table 5. P. balsamifera had a uniformly high number of seeds per carpel, while P. deltoides var. occidentalis had a variable smaller number of

seeds per carpel. The Grand Beach variants had a mean value higher than that for P. deltoides var. occidentalis, but it did not approach the value that might be expected for hybrids of P. balsamifera and P. deltoides var. occidentalis. The Delta and Oak Lake variants both had values smaller than either P. balsamifera or P. deltoides var. occidentalis. Two trees from each of the Delta and Oak Lake areas failed to produce mature catkins and seeds as catkins abscissed in the early stages of development. This condition was not noted in any of the other species. The apparent reduction in seed set for most of the variants would agree with other studies that have demonstrated sterility among hybrid individuals. Due to the small number of collections and large variation within collections more collections are required to confirm these results.

Table 5. Number of seeds per carpel in P. balsamifera, P. deltoides var. occidentalis and three variant collections.

Collection	N	Range	Mean
P. balsamifera	12	11.7-16.5	13.4
P. deltoides var. occidentalis	10	0 - 8.2	3.6
Populus - Delta	13	0 - 1.5	.7
Populus - Oak Lake	18	.5 - 4.2	2.5
Populus - Grand Beach	7	2.0 - 6.9	4.2

The percent germination and percent weak seedlings for P. balsamifera, P. deltoides var. occidentalis, and three variant collections are presented in Table 6.

Table 6. Percent seed germination and percent weak seedlings for *P. balsamifera*, *P. deltoides* var. *occidentalis* and three variant collections.

Collection	N	Percent germination	Percent weak seedlings
P. balsamifera	6	100.0	4.5
P. deltoides var. *occidentalis*	6	99.6	5.0
Populus - Delta	4	97.5	3.8
Populus - Oak Lake	18	99.2	5.7
Populus - Grand Beach	6	90.0	30.0

Only the Grand Beach variants showed a marked decrease in the percent germination and percent weak seedlings when compared with the parental species.

Although some apparent hybrids failed to produce mature catkins and some showed an apparent reduction in seed set, the data would indicate that large numbers of viable seed could still be produced. These observations demonstrate the possibilities for the complex intergradation of the species over a period of time. An example of this intergradation can be seen in the Delta variants which show a variation in leaf forms from those similar to *P. balsamifera* to those that approach *P. deltoides* var. *occidentalis*. Löve and Löve (15) have shown that a number of vegetation zones exist along the forested ridge at Delta. These zones offer a number of sites for the various segregation products resulting from complex hybridization. This could favor the development of a complex hybrid swarm in this area.

Chemotaxonomic Study

A modification of the gas chromatographic techniques used by Bolan and Steele (6) for the separation of phenolic glycosides in Salix, was found suitable for chemotaxonomic studies in Populus. The gas chromatographic technique used resulted in good separation of a number of compounds in extracts from the genus Populus. Although none of the compounds were identified chemically it is believed that most if not all of the peaks represent phenolic glycosides compounds. The five phenolic glycoside reference compounds each produced a single peak in the chromatogram.

Two chromatograms of each of the four native species are shown in Figures 38-45. Chromatograms of four variants are shown in Figures 46-49. All chromatograms shown contain tremuloidin as a reference compound.

Eleven distinct peaks, in addition to the reference compound, tremuloidin, (Peak #6) were observed in the chromatograms. The first five peaks, when present, were very distinct. The bases of peaks 7, 8, and 9 overlapped to some extent and this same condition occurred for peaks 10 and 11. The retention values for each peak relative to tremuloidin were recorded (Appendix, Table 7) and this served to identify each peak. A number of smaller peaks showed up on each chromatogram, but these were often variable and of minor occurrence.

The chromatograms of P. grandidentata (Figures 38 and 39) were distinct from those of other species. The chromatograms of P. grandidentata had large quantities of compounds 7, 8, and 9 in distinct proportions. Compound 7 was always present in the largest amount while compound 9 was present in the smallest amount. Neither P. tremuloides (Figures 40 and 41), P. balsamifera (Figures 42 and 43), or P. deltoides var. occidentalis (Figures 44 and 45) had compounds 7, 8, and 9

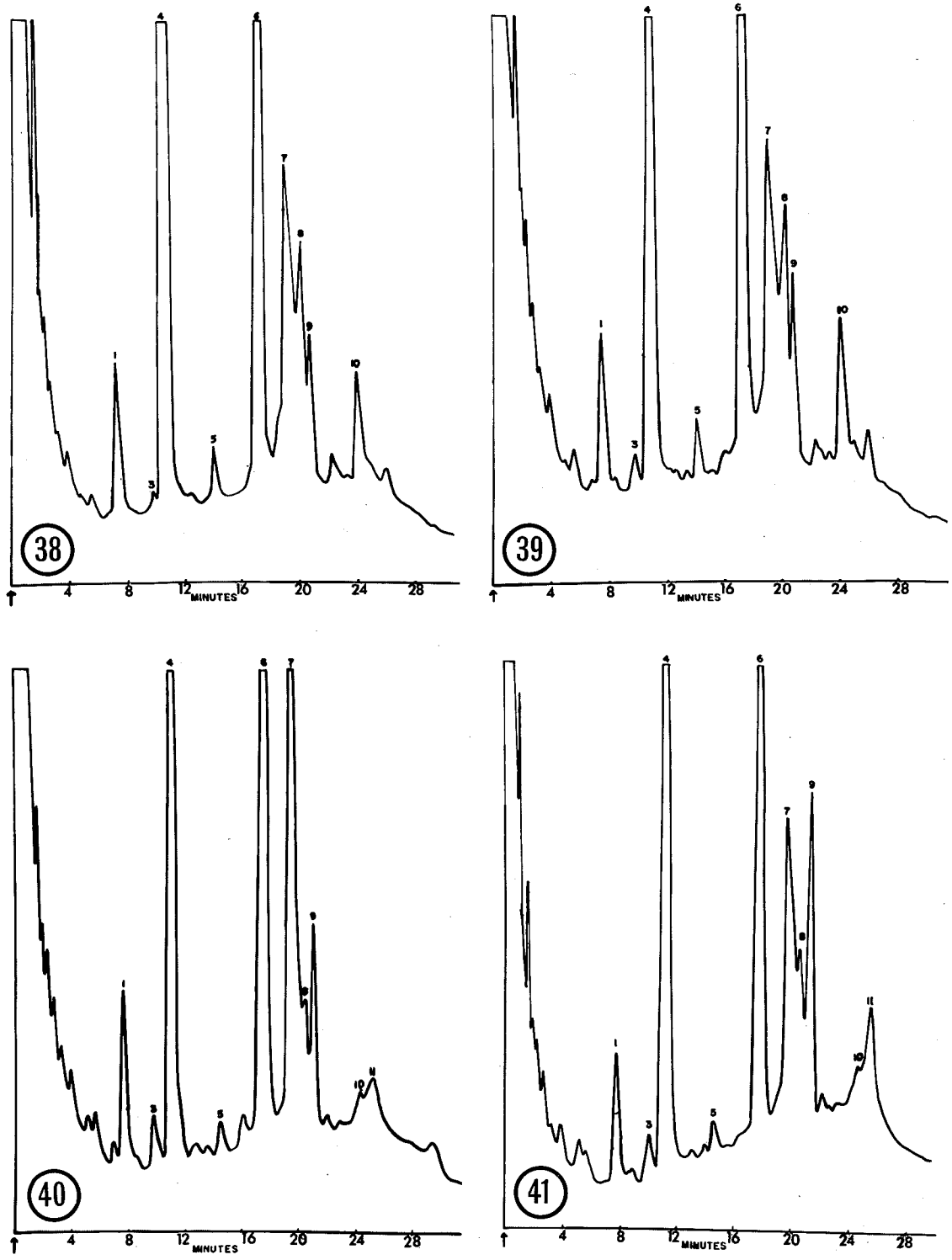


Figure 38. Chromatogram of P. grandidentata from Betula Lake.
Figure 39. Chromatogram of P. grandidentata from Rennie.
Figure 40. Chromatogram of P. tremuloides from Delta.
Figure 41. Chromatogram of P. tremuloides from Grand Beach.

in the same relative proportion as in P. grandidentata. Although some of the variant chromatograms (Figures 46-49) had compounds 7, 8 and 9 in the same relative proportion as P. grandidentata, they could be identified as the amount of compound 9 was very small in proportion to compounds 7 and 8. P. grandidentata chromatograms showed larger amounts of compound 10 than in any of the other chromatograms.

P. tremuloides chromatograms (Figures 40 and 41) always had less compound 8 than either 7 or 9. This was a distinctive feature of chromatograms of this species. This similarity of P. tremuloides and P. grandidentata chromatograms supports the natural taxonomic classification, based on morphology, which places them in the same section of the genus Populus.

P. balsamifera chromatograms (Figures 42 and 43) showed large amounts of compound 5 which did not occur in large amounts in either of P. grandidentata, P. tremuloides, or P. deltoides var. occidentalis. The presence of compound 12 and the relative proportions of compounds 7, 8 and 9 were distinctive of P. balsamifera.

The chromatograms of P. deltoides var. occidentalis (Figures 44 and 45) could easily be identified as they lacked large amounts of compound 5, 7, 8, 9, 10, 11 and 12.

The fact that the chromatograms of P. balsamifera appeared more similar to P. tremuloides and P. grandidentata, than to P. deltoides var. occidentalis would appear to conflict with the morphological and phenological results that would show that P. balsamifera is more like P. deltoides var. occidentalis than P. tremuloides. Chromatograms of P. deltoides var. occidentalis were distinctly different from those for the other species. This would lend support to the taxonomic classification that places P. deltoides var. occidentalis in a different section

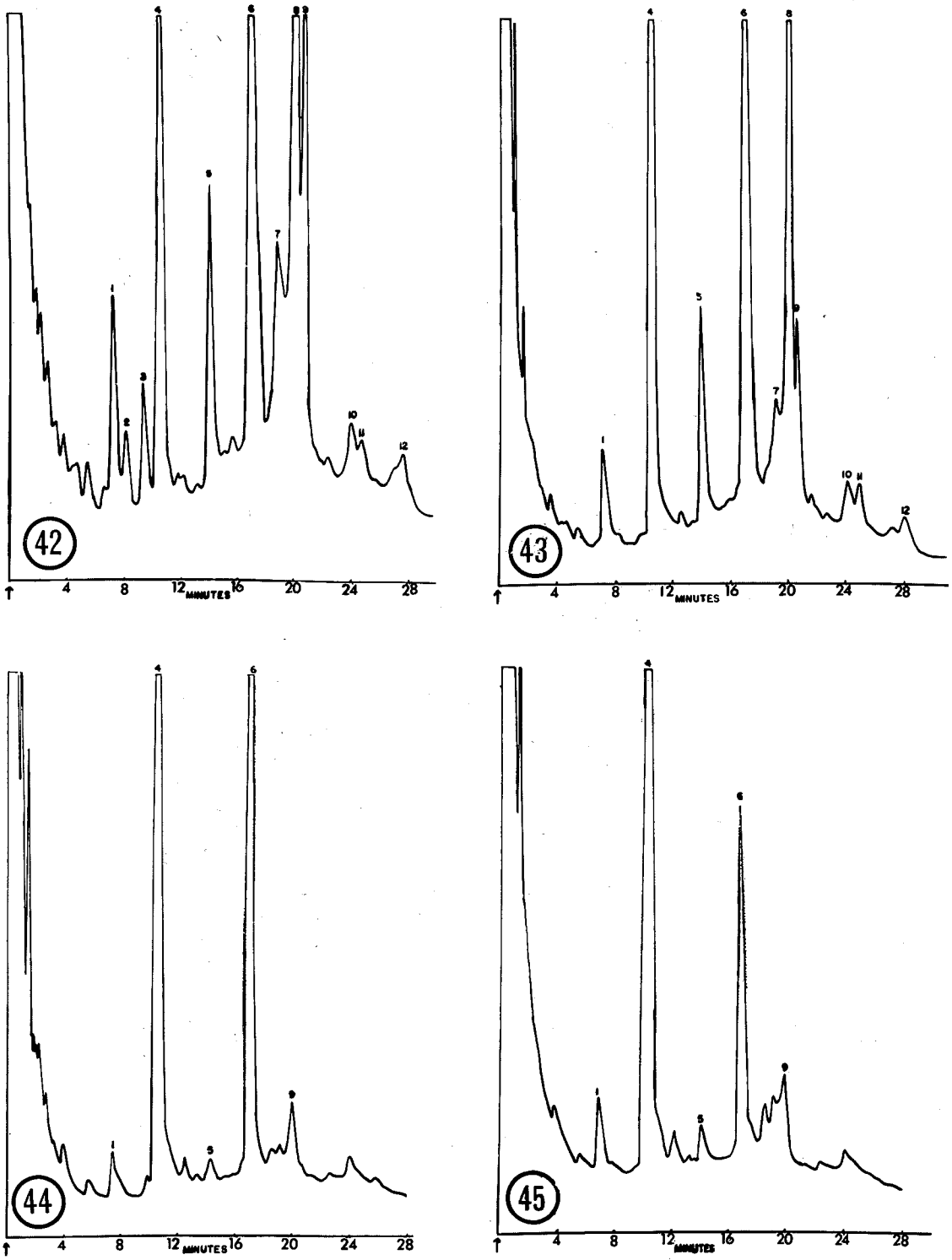


Figure 42. Chromatogram of P. balsamifera from Delta.
Figure 43. Chromatogram of P. balsamifera from Grand Beach.
Figure 44. Chromatogram of P. deltoides var. occidentalis from Delta.
Figure 45. Chromatogram of P. deltoides var. occidentalis from Grand Beach.

of the genus.

The chromatograms of the variants examined were quite similar. A number of differences in the size of the peaks could be used to identify the variant chromatograms (Figures 46-49) as distinct from those for the four species (Figures 38-45). Compounds 1-4 were not very useful in identifying variant chromatograms. Compound 5 was present in the variants in amounts greater than in P. grandidentata, P. tremuloides, and P. deltoides var. occidentalis. In three cases the variants had less compound 5 than P. balsamifera, but in one case (Figure 47) the variant was similar to P. balsamifera. The large amounts of compound 5 in Figure 48 may indicate that this variant has resulted from back-crossing to P. balsamifera. The occurrence of large amounts of compound 5 in the variants would indicate that P. balsamifera is one of the parents. Compounds 7 and 8 were present in greater amounts than in P. deltoides var. occidentalis, but were usually present in smaller amounts than in either the P. grandidentata and P. tremuloides, or the P. balsamifera. All variant chromatograms showed small amounts of compound 9 and no apparent trace of compounds 11 or 12.

The variant chromatograms appeared to be intermediate between those for P. balsamifera and those for P. deltoides var. occidentalis; however, the variant chromatograms showed smaller amounts of compound 8 and 9 than expected. The variant chromatograms were closely alike for compound 8 and 9 thus supporting the morphological and phenological data that would indicate that all natural variants between native species of Manitoba are of one combination.

Even though chromatograms were made of trees from widely separated regions of southern Manitoba, it was possible to identify both the

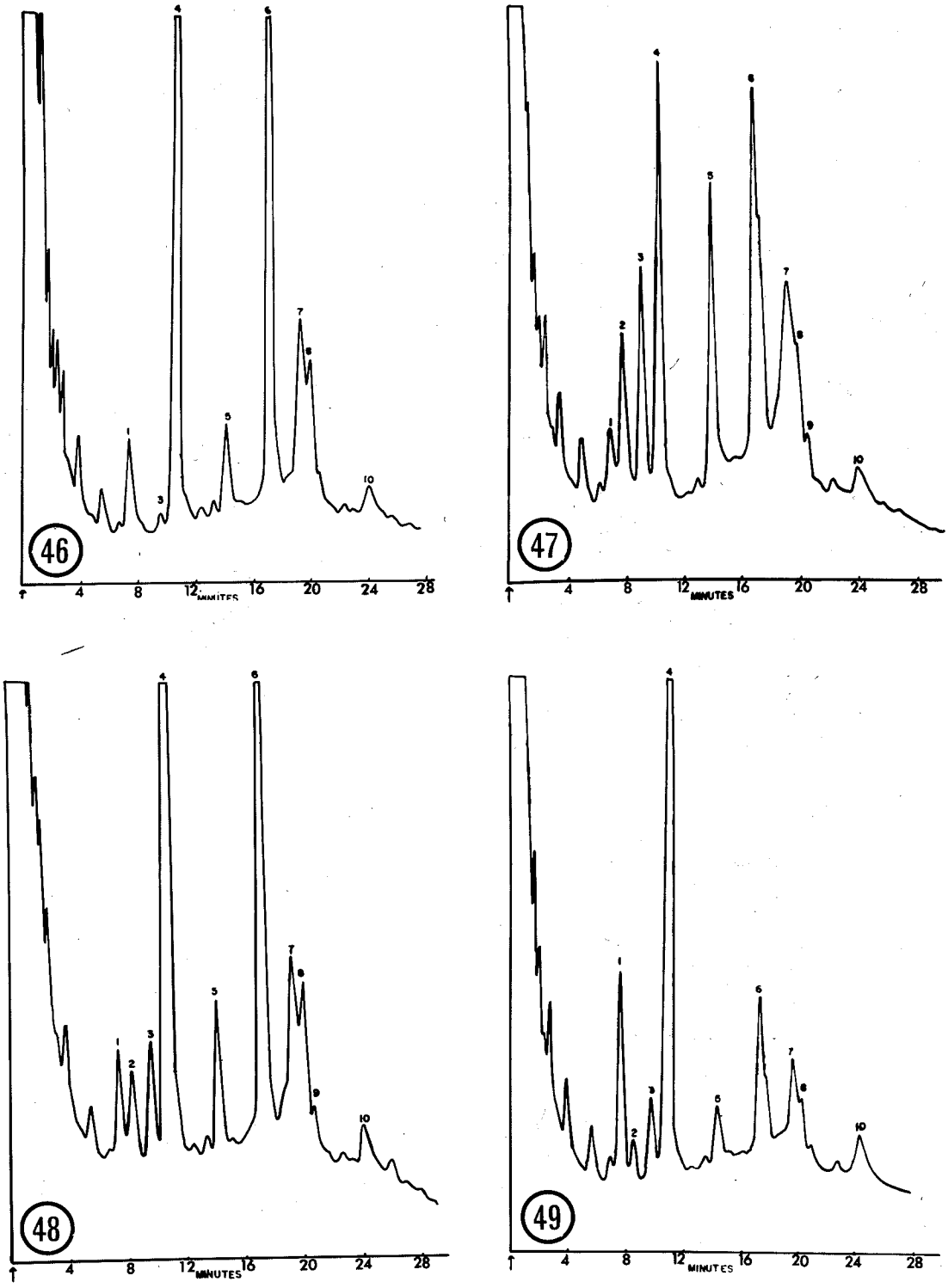


Figure 46. Chromatogram of P. Jackii 'Northwest'.
Figure 47. Chromatogram of Populus #110 from Grand Beach.
Figure 48. Chromatogram of Populus #210 from Oak Lake.
Figure 49. Chromatogram of Populus #310 from Delta.

species and the variants. In this limited study, it was not found possible to show any relationship between the chromatogram and the sex of the tree. This evidence agrees with Thieme (24) who found that a general dependence of glycoside content on the sex of the plant could not be found.

Most of the chemical differences found between species involved quantitative variation, although some qualitative differences did exist. The importance of quantitative differences in identifying species and hybrids explains the difficulties that have been found by Ronald et al. (19) when attempting to use only paper chromatography. The more refined methods of gas chromatography enables one to accurately measure these quantitative differences.

Gas chromatography of compounds in Populus and Salix may prove to be as useful as found in Picea by von Rudloff (25,26,27). It is possible that only a small part of the chemical variation of the four Populus species has been sampled. Studies of more distant collections may reveal more chemical variation some of which may have definite taxonomic implications. The status of the two subspecies of P. balsamifera and the two varieties of P. deltoides could possibly be resolved as both are based on minor morphological differences and geographic distribution.

SUMMARY AND CONCLUSIONS

Species and variants of the genus Populus, native to southern Manitoba, were collected in 1967 and 1968. The distribution of P. grandidentata, P. deltoides var. occidentalis and native variants was plotted.

Twenty-four morphological characters were studied from collections of winter twigs, female catkins and foliage. The range in variation of these characters was plotted.

Hybrid index values and pictorialized scatter diagrams prepared for the Delta collections and the cultivar 'Northwest', indicate that the majority of the variants appear to be F_1 hybrids and that most of the backcrossing and segregation has been towards P. balsamifera. Thus, over a period of time, genes from P. deltoides var. occidentalis would appear to be introgressing into P. balsamifera.

The dates of anthesis and seed shedding for P. tremuloides differed from the dates for P. balsamifera, P. deltoides var. occidentalis and the variants. The overlapping dates of anthesis for P. balsamifera and P. deltoides var. occidentalis explains the occurrence of numerous apparent hybrids where these two species grow together.

A chemotaxonomic technique, employing gas chromatography, was used to identify both species and variants. Eleven compounds were observed in chromatograms and of these several were of taxonomic importance.

On the basis of evidence obtained from morphological and phenological studies, and chemical taxonomy, it appears that all native variants that were collected in southern Manitoba have resulted from hybridization between P. balsamifera and P. deltoides var. occidentalis, and should therefore be named Populus Jackii Sarg. This is not in agreement with Boivin (5) who listed two other hybrid combinations as

native to Manitoba. There appears to be little firm evidence of hybridization between P. tremuloides and P. deltoides var. occidentalis. Specimens of this combination, collected in Manitoba and loaned from the Plant Research Institute, were examined morphologically and would appear to be hybrids of P. balsamifera and P. deltoides var. occidentalis. A number of characters occurring in the 'Northwest' cultivar and other variants and used in preparing the hybrid index and pictorialized scatter diagram were found only in P. balsamifera and the hybrids. Natural hybrids between P. tremuloides and P. balsamifera were not found in Manitoba. This agrees with the results obtained by Brayshaw (9) in Alberta where he found no direct hybrids between P. tremuloides and P. balsamifera. Two specimens from Manitoba identified as hybrids of P. tremuloides and P. balsamifera and loaned from the Plant Research Institute were examined. The identification of one of these has been revised to P. tremuloides and the other to P. Petrowskyana. No hybrids of P. grandidentata were found.

The cultivar 'Northwest' appears to have resulted from hybridization of P. balsamifera and P. deltoides var. occidentalis.

LIST OF REFERENCES

1. Alston, R. E. and B. L. Turner. 1962. New technique in analysis of complex natural hybridization. Proc. Natl. Acad. Sci. U.S.A. 48: 130-137.
2. Anon. 1956. Development of Horticulture on the Canadian Prairies: An Historical Review. Compiled by the W.C.S.H.
3. Barnes, V. B. 1961. Hybrid aspens of the Lower Peninsula of Michigan. Rhodera 63: 311-324.
4. Boivin, B. 1966. Enumeration des Plantes du Canada. II. Lignidees. Naturaliste Can. 93: 371-437.
5. _____. 1967. Flora of the Prairie Provinces. Saskatchewan Natural History Society.
6. Bolan, M. and J. W. Steele. 1968. Phytochemistry of Salix species. I. A gas liquid chromatographic procedure for the separation and identification of phenolic glycosides. J. Chromatog. 36: 22-30.
7. Boritz, S. 1962. Paperchromatographische differenzierung einizer Arten und Sorten der Galtung Populus. Der Zuchter 32: 24-33.
8. _____. 1963. Identification of Salicaceae and other forest trees by paperchromatographic separation of the fluorescent constituents. Bull. de L'Acadamie Colonaise des Sciences XI: 549-554.
9. Brayshaw, T. C. 1965a. Native poplars of southern Alberta and their hybrids. Canada Dept. of Forestry Publ. #1109.
10. _____. 1965b. The status of black cottonwood (Populus trichocarpa Torrey and Gray). Can. Field-Naturalist 79: 91-95.

11. Crovello, T. J. 1966. Quantitative taxonomic studies in the genus Salix. Ph.D. Thesis Univ. Calif., Berkeley.

12. Davis, P. H. and V. H. Heywood. 1965. Principles of Angiosperm Taxonomy. Oliver and Boyd, Edinburgh and London.

13. Fisher, M. J. 1928. The morphology and anatomy of the flowers of the Salicaceae. Amer. Jour. Bot. 15: 372-394.

14. Jackson, V. W., J. F. Higham, H. Groh and C. W. Lowe. 1962. Checklist of Manitoba Flora. Natural History Society of Manitoba.

15. Löve, A. and D. Löve. 1954. Vegetation of a Prairie Marsh. Bull. Torrey Bot. Club 81: 16-34.

16. Maini, J. S. 1966. Apical growth of Populus spp. I. Sequential pattern of internode bud and branch length of young individuals. Can. J. Botany 44: 615-622.

17. Moss, E. H. 1938. Longevity of seed and establishment of seedlings in species of Populus. Bot. Gaz. 99: 529-542.

18. Nagaraj, M. 1952. Floral morphology of Populus deltoides and P. tremuloides. Bot. Gaz. 114: 223-243.

19. Ronald, W. G., L. M. Lenz and W. A. Cumming. 1968. Unpublished results.

20. Scoggan, H. J. 1957. Flora of Manitoba. National Museum of Canada, Bulletin 140.

21. Smith, E. C. 1943. A study of cytology and speciation in the genus Populus L. Jour. Arn. Arb. 24: 275-305.

22. Steele, J. W., M. Bolan and R. C. S. Audette. In Press.

23. Stettler, R. F. 1968. Irradiated mentor pollen: Its use in remote hybridization of Black Cottonwood. Nature 219: 746-747.

24. Thieme, H. 1965. The phenolic glycosides of Salix. Die Pharmazie 20: 570-574.

25. von Rudloff, E. 1967a. Chemosystematic studies in the genus Picea (Pinaceae). I. Introduction. Can. J. Botany 45: 891-901.

26. _____. 1967b. Chemosystematic studies in the genus Picea (Pinaceae). II. The leaf oil of Picea glauca and P. Mariana. Can. J. Botany 45: 1703-1714.

27. _____, and M. J. Holst. 1968. Chemosystematic studies in the genus Picea (Pinaceae). III. The leaf oil of a Picea glauca x mariana hybrid (Rosendahl spruce). Can J. Botany 46: 1-4.

APPENDIX

WINTER KEY TO *POPULUS* OF SOUTHERN MANITOBA

a. Terminal vegetative buds, on short growing laterals, less than 9 mm in length; flower buds less than 12 mm in length; stamen number less than 15; catkin bracts with less than 10 divisions; female flowers more than 85 per catkin; male flowers more than 95 per catkin.

b. Buds heavily pubescent; non resinous, appearing downy white .... *P. grandidentata*

bb. Buds glabrous, usually partly covered with a hard brown resin, shiny brown ..... *P. tremuloides*

aa. Terminal vegetative buds, on short growing laterals, greater than 12 mm in length; flower buds greater than 12 mm in length; stamen number greater than 20; catkin bracts with greater than 10 divisions; female flowers less than 70 per catkin; male flowers less than 95 per catkin.

c. One year old twigs yellow or tawny yellow in color, glabrous; flower buds with 6 or more scales; buds pubescent especially at base ..... *P. deltoides* var. *occidentalis*

cc. One year old twigs brownish in color, usually pubescent; flower buds with less than 6 scales; buds pubescent.

d. One year old twigs light brown in color, usually pubescent; flower buds with 4 or 5 scales ..... *P. Jackii*

dd. One year old twigs dark reddish brown in color, pubescent; flower buds with 3 or 4 scales.

e. Flower buds lightly pubescent ..... *P. balsamifera*

ee. Flower buds heavily pubescent ..... *P. balsamifera* var. *subcordata*

SUMMER KEY TO POPULUS OF SOUTHERN MANITOBA

a. Leaves round in outline, briefly acuminate leaf apex.

b. Leaves finely toothed with more than 20 teeth per side .....

P. tremuloides

bb. Leaves coarsely toothed with less than 20 teeth per side .....

P. grandidentata

aa. Leaves deltoid to lanceolate, acuminate to attenuate leaf apex.

c. Leaves heavily whitened and often with rusty blotches, on dorsal surface.

d. Leaves lanceolate; leaf base round to cuneate .....

P. balsamifera

dd. Leaves orbicular-lanceolate; leaf base cordate .....

P. balsamifera var. subcordata

cc. Leaves green or greenish-white on dorsal surface.

e. Leaves deltoid, coarsely toothed, green on dorsal surface; petiole strongly compressed..... P. deltoides var. occidentalis

ee. Leaves deltoid to lanceolate, finely toothed to moderately coarse toothed, greenish-white on dorsal surface; petiole slightly compressed ..... P. Jackii

Table 7. Relative retention values of trimethylsilylated compounds with respect to trimethylsilyl tremuloidin.

Compound	OV-1 column
1	0.44
2	0.49
3	0.56
4	0.62
5	0.83
7	1.10
8	1.17
9	1.21
10	1.39
11	1.43
12	1.59
Retention time of trimethylsilyl tremuloidin (min.)	17.6
Carrier gas flow (ml/min.)	45
Column temperature (°C)	185-250
Inlet temperature (°C)	250
Detector temperature (°C)	300
Column length (ft.)	6