

STUDIES IN THE COMPARATIVE ANATOMY OF THE VESSEL
ELEMENTS OF THE SECONDARY XYLEM OF ACER NIGRUM,
PLATANUS OCCIDENTALIS, AND RHODODENDRON MAXIMUM

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Introduction

There is a need for a complete anatomical study of the secondary xylem tissue of many vascular species today to corroborate the work which has been done in classifying plants using floral anatomy and other anatomical characteristics. That work which has been reported is, for the most part, of such a nature as to permit speculation of those suspected characteristics which have for their basis merely incomplete anatomical evidence. It is generally believed that all secondary xylem vessel elements are essentially similar anatomically regardless from which portion of the secondary xylem they are obtained. The basis for this belief has been data obtained from the study of vessel elements in the first up to the fifth (infrequently the tenth) growth rings. Very little work has been done with growth rings beyond this point - in fact there are only a very few instances where work has been done beyond the fifth growth ring. This belief that all secondary xylem vessel elements are essentially similar may be correct, yet the corroborative evidence is lacking. It is therefore believed that only after a complete anatomical study has been made of a specific tissue can there be made qualified statements as to the characteristics of the tissue as a whole.

Literature Review

The need for anatomical evidence to complete or support other data in classifying vascular plants is unquestionably great. Such evidence would be invaluable to taxonomists to support the known morphological characteristics of the various tissues.

A tissue which is particularly valuable, in reference to dicotyledons, with reference to evolutionary specialization is secondary xylem tissue. Specialization appears to occur in the outermost or youngest secondary xylem and proceed inward towards the primary xylem (Foster, 1949). Such a characteristic, then, attributable to the youngest secondary xylem should be studied for several reasons:

1. Vessel elements are dead at maturity and therefore can be handled without fear of disrupting or destroying necessary cell contents.
2. There should be present a gradient of characteristics from the primary xylem vessel elements to the youngest secondary xylem vessel elements; a gradient showing less specialized to more highly specialized characteristics.
3. Xylem tissue is relatively easy to obtain and can be stored without fear of disrupting the cells by excessive dehydration.
4. Vessel elements are quite readily freed from tissue by maceration and they can be stained using safranin, methyl green, or crystal violet.

The anatomical work completed during the latter half of the nineteenth century and the first few years of the twentieth century has been well summarized by Solereder (1908). His compilation of data concerning secondary xylem ("wood" in his work) is brief and of a generalized nature. However, the data presented is, for the most part, accurate. There are mentioned reports of the diverse appearance of growth rings caused by variable widths of vessel elements and the arrangement of the vessel elements. Solereder also reported the presence of scalariform and simple perforations at opposite ends of the same vessel element; a characteristic of the genus Platanus, and tertiary spiral thickenings in the walls of vessel elements of Acer and Rhododendron.

A rather complete report of all the work that has been done concerning the anatomy of plants was compiled by Metcalfe and Chalk (1950). These two volumes contain an extensive bibliography of the more important botanical workers. Specific reference is made concerning the vessel elements comprising the xylem of all of the higher plants. To be sure, these data are brief and general - reference being made to the tissue as a whole. These data are, however, indicative of the known anatomical characteristics of the components comprising the xylem.

An approach to the anatomical study of secondary xylem tissue as a whole was made by Prichard and Bailey (1916). These men worked primarily with xylem fibers and infrequently with vessel elements. Various anatomical features of the fibers and vessel elements were measured at random from each of the growth rings. Statistically, the data obtained were not entirely sound because fibers are difficult cells with which to

work and they do not exhibit the many and varied phylogenetic characteristics as do vessel elements. The number of fibers and vessel elements measured was not sufficient to assure the validity of the work. Nevertheless, such an approach would serve to indicate that variations are present or are not present. If it appeared that variations were present, it would indicate that more detailed work should be done to clarify the existing knowledge of the tissue.

The evolutionary characteristics shown by secondary xylem tissue have made this part of the plant a particularly fruitful field for anatomical investigation. Data obtained from any of the vascular plants of a particular class seem to bear-out the fact that tissue specialization forms a gradient from the less specialized to the more highly specialized. With xylem vessel elements, specific anatomical characteristics of the individual cell have been studied for degree of evolutionary change. Bailey and Tupper (1918) presented data which significantly showed the relationships between specialization in the various types of lateral wall pitting - scalariform pitting being primitive and opposite pitting the most highly advanced. That vessel elements have been derived from tracheids is now an almost universally accepted theory. Considerable work commencing with Boodle and Worsdell (1894), continuing with Thompson (1918, 1923) and Bliss (1921), and being summarized by Frost (1930b) and Bailey (1944b) has conclusively proven the validity of the theory. Consequently, those vessel elements retaining many tracheid characteristics are considered more primitive than those not retaining a great many tracheid characteristics.

The evolutionary development of the vessel element of dicotyledonous wood has been summarized quite fully in three papers by Frost (1930a, 1930b, and 1931). His conclusions were based upon the voluminous data obtained in the 40 odd years preceding his first publication. Furthermore, his conclusions are considered today to be the more important of the tenets used as criteria in determining primitive or advanced characteristics.

Cox (1948) proposed another view to the list of distinguishing characteristics - that of vessel element length-width ratio. An element having a low ratio being more advanced than one with a high ratio.

Petersen (1953) compiled a list of twenty-two lines of structural specialization which incorporates the majority of the features now in use in determining the advanced or primitive characteristics of secondary xylem.

The literature is conspicuously devoid of data pertaining to the anatomy of vessel elements in the growth rings beyond the fifth and infrequently the tenth growth rings. It is therefore necessary to study the anatomy of the components of the growth rings beyond the fifth ring to completely clarify the anatomy of the secondary xylem in vascular plants.

Some preliminary work, Holden (1912), Baird (1915), and Cox (1948), has been done with the three genera used in this study. However, nothing could be found to indicate that specific studies have been devoted to the outer portions of the secondary xylem.

Objectives

A. Object of Investigation

Investigation of the botanical literature failed to reveal any reports on studies devoted to secondary xylem vessel elements of different growth ring areas in the same species. Furthermore, nothing could be found which correlated the various phylogenetic features of vessel elements from growth ring areas of xylem tissue which were smaller in width on one side of a limb or branch than on the other side. The difference in width, (which will be called herein the expanded xylem area and compressed xylem area), might possibly be caused by increased cambial activity resulting in the formation of many more cells and subsequently an increase in size of the growth ring on one side. Physiological conditions such as light intensity, light duration, temperature etc. might be factors involved in the increase in cambial activity. On the other hand, difference in width between expanded and compressed growth rings may be due to a difference in size of xylem elements produced by the cambium in the two areas. Therefore, to investigate the possibility that there may be significant differences in vessel element structure in different growth ring areas or to corroborate the accepted thesis that xylem elements are homogeneous in structure throughout the wood of a given species, the following outline of investigation was established:

1. Wood, with at least ten growth rings, was obtained from a species of three different genera (viz. Acer nigrum, Platanus occidentalis, and Rhododendron maximum), macerated, placed on slides, and stained.

2. The slides were examined microscopically to determine:

a. Any significant difference in the vessel element structure between growth ring areas (i.e. compressed xylem area versus expanded xylem area) of the same plant.

b. Any significant difference in vessel element structure between growth rings from the same area of the same plant. The growth rings were grouped in series of five growth rings each (e.g. Group I, growth rings 1-5, Group II, growth rings 6-10, Group III, growth rings 11-15 (11-12 in Platanus), and Group IV, growth rings 16-20).

c. Any significant differences between vessel element structure of the same group but from a different area (i.e. comparison of vessel elements from Group I of compressed xylem area and from Group I of expanded xylem area, etc.)

It is believed that the above outline may be helpful in the scheme of systematics - either correlating what is now known or helping in revising the system more accurately.

B. Materials and Methods

The material for this study consisted of portions of woody stems having secondary xylem growth with at least ten annular growth rings.

Specimens used were obtained from the following areas:

1. Platanus occidentalis - large branch from a well developed tree growing in the southwest corner of the V.P.I. campus.
2. Acer nigrum - large branch from a well developed tree growing adjacent to the entrance of Price Hall, V.P.I. campus.

3. Rhododendron maximum - branch from the area adjacent to the Mountain Lake (Virginia) Biological Station.

The material was brought to the laboratory, sawed into one-foot lengths, stripped of bark, and allowed to dry at room temperature for approximately one week.

After drying, one inch thick discs were cut perpendicular to the main axis from a randomly selected one foot section of each of the three species. The discs were sanded to facilitate counting of the growth rings. The growth rings were counted visually with the aid of a 10X hand lenses. Approximate ages of the sections of the species used were ascertained as follows:

1. Platanus occidentalis - 12 years
2. Acer nigrum - 20 years
3. Rhododendron maximum - 50 years

It was noted when counting the growth rings that the rings were uneven in thickness - one side appearing to be compressed - the other expanded. This led to the treatment of the two areas separately to determine if there would be any difference in the anatomical structure of the two areas.

The discs from each of the three species were further cut-up into blocks; each block encompassing five growth rings. Blocks were cut both from the compressed growth ring and the expanded growth ring areas.

Tissue maceration was accomplished using Jeffrey's technique (Johansen, 1940). Small chips were shaved or cut from each of the blocks (which encompassed five growth rings) cut from the three species and

boiled in distilled water to remove the air in the tissue. The boiled chips were placed in vials and covered with a 1:1 dilution of Jeffrey's maceration fluid and allowed to stand in an oven at about 60°C from 24-36 hours. Separation of the individual cells was facilitated by crushing the tissue gently with the rounded end of a solid glass rod. There was considerable variation in time and strength of maceration fluid required to separate the material even in different portions of the same species. Occasionally, the 1:1 dilution proved to be too strong and complete dissolution of the material apparently ensued. For the bulk of the material, however, a 1:1 dilution proved to be satisfactory. After separation of tissues, the material was repeatedly washed with distilled water to remove all of the maceration fluid. Material was mounted immediately after washing on standard glass slides. Excess material was stored in a 1:1:1 mixture of glycerin, 70% ethyl alcohol, and distilled water.

The technique used in preparing slides was that of Cox (1948). A slide was prepared with a thin film of Haupt's gelatin adhesive over which was placed 3 drops of 8% formalin. To this preparation, 3 drops of water containing an abundance of the macerated material was added. A smear was made over the slide and then allowed to dry on a slide warmer. Slides prepared by the above technique were easily handled in using Coplin jars and standard staining procedures. The slides were stained with safranin for 24 hours and mounted in balsam following standard techniques (Johansen, 1940). On examination of slides prepared from Rhododendron maximum, it was discovered that the vessel elements stained

weakly or not at all whereas the fibers stained a vivid red. Variations in staining time and dehydration time were attempted with little success. Inspection of the stored macerated material indicated an abundance of vessel elements. Several wet mounts were made and found to be very good for use in measuring the vessel elements. Several drops (5-10) of safranin were added to the vials in which the macerated material was stored at least 12 hours prior to use to provide a slightly darkened background. This technique proved to be excellent for measuring and inspecting the elements provided there was an abundance of material. Such a technique could be used as a supplement to but not as a replacement for the permanent slide technique.

The criteria used in comparative studies of secondary xylem tissue have been greatly expanded during the past forty years. Papers by Bailey and Tupper (1918), Bailey (1944), Frost (1930a, 1930b, and 1931), Cox (1948), and Petersen (1953) are among the more important studies listing and employing the tenets used as criteria for comparative anatomical studies.

The criteria used for anatomical comparisons in this study were those considered to be the more important of the more than twenty-five lines of anatomical specialization. The criteria were as follows:

1. Vessel Element Dimensions:

- a. Average vessel element length - vessel elements were measured encompassing the over-all length of the vessel element. The measurements included the vessel element "tails" as suggested by Chalk (1934) because the "tails"

are a legitimate portion of the vessel element arising from the cambial initial. Longer vessel elements are believed to be phylogenetically more primitive than shorter derived elements. Averages were computed from a minimum of 100 measurements of individual cells selected at random from a slide of macerated material or from a wet mount.

- b. Average vessel element diameter - vessel elements were measured at a point where each cell appeared to be the widest. Larger diameters are considered to be more advanced phylogenetically than smaller diameters. Averages were computed from a minimum of 100 random measurements.
- c. Ratio of average vessel element length to average vessel element diameter - ratios were computed by dividing the average vessel element diameter into the average vessel element length. This criterion was originally proposed by Cox (1948). The higher the ratio between the two vessel element dimensions, the more primitive phylogenetically is the particular species. A low ratio is indicative of an advanced phylogenetic condition.

2. Vessel Element Perforation Plates - Vessel elements were viewed so as to determine the type of perforation plate present at the ends of each cell. Scalariform perforation plates were listed noting the number of cross bars across the perforated area. Simple perforations are believed to be more advanced phylogenetically than scalariform perforations.

Similarly, scalariform perforations with few bars are believed to be more advanced than those with many bars.

3. Vessel Element Side Wall Pits - Pits present in the side walls of the vessel elements were noted. Pits were classified as follows - the first listed believed to be the more primitive phylogenetically; the last listed the more advanced:

- a. Scalariform
- b. Elongate
- c. Elliptic
- d. Alternate
- e. Opposite

4. Vessel Element End Wall Angle - End wall angles were ascertained visually with no mechanical measuring device. End wall angles were computed not entirely accurately using the horizontal as an angle of 90° and the vertical (the vertical assumed to be that angle perpendicular to the horizontal) as an angle of 0° . A highly inclined end wall is believed to be more primitive phylogenetically than one of a transverse or 90° angle.

5. Tertiary Thickening of Vessel Element Wall - Walls of the vessel elements were noted for indications of tertiary thickenings. Tertiary thickenings are believed to be an indication of an advanced condition whereas an absence of tertiary thickenings is believed to be a primitive condition.

The data obtained from the anatomical study of secondary xylem

vessel elements were to be used to corroborate the existing thesis that xylem elements are homogeneous in structure throughout the wood of a given species or to demonstrate that differences in xylem elements do exist in different growth ring areas of a given species.

It is further believed that evidence of an anatomical nature obtained in this study will verify classifications based on morphological characteristics or differ significantly to be of aid in revising the system more accurately.

Any anatomical differences encountered in compressed growth rings and expanded growth rings may be accountable to physiologic causes and therefore warrant an intense study of this xylem peculiarity.

The three genera used in this study were chosen primarily because of the availability of the material and secondarily because one of the species is of a diffuse porous wood texture (Rhododendron), one of the species is of a ring porous wood texture with certain characteristics of an anatomically herbaceous type xylem (Platanus), and one of the species is of a ring porous wood texture (Acer). It is believed that such a cross section of characteristics would serve to demonstrate the feasibility of utilizing anatomical data from all species for correlating morphological and physiological data.

Results and Discussion

The three species studied will be discussed separately and under individual headings, since the three species are unrelated and far apart taxonomically.

Acer nigrum

The genus Acer is a member of the family Aceraceae whose members are characterized as trees or shrubs with watery saccharine sap. Flowers are bisexual or more commonly unisexual, hypogynous or perigynous, in corymbs, racemes, or panicles, and with 4-5 sepals and 4-5 petals (occasionally without petals). Stamens 4-10, generally 8, inserted on the end of a disc. Anthers 2-celled. Ovary superior, 2-locular, 2 lobed; 2 ovules in each locule. Pistils 1 with 2 styles. The fruit is a samaroid schizocarp splitting into 2 one-winged mericarps. Seed lacks endosperm.

Anatomically, the family is characterized by Solereder (1908) and Metcalfe and Chalk (1950) as follows: (1) simple or porous perforations. (2) spiral tertiary thickenings in walls of vessel elements. (3) vessels solitary or in groups of 2-3 cells. (4) medullary rays narrow and (5) bordered pits in vessel element side walls.

Generally speaking, the anatomical characteristics measured and viewed in this study were the same as those characterizing the family. In addition to those characteristics listed before, the following characteristics were found to be present: (1) vessels frequently in groups of 4-6 cells. (2) end wall angle of vessel elements 45° - 80° . (3) lateral wall pitting of vessel element walls generally alternate; infrequently

opposite. The above characteristics serve to corroborate the accepted phylogenetic position of the family since they indicate generally more advanced anatomical characteristics.

The various anatomical characteristics of this species are listed in Table I. There appears to be a small deviation in the lengths of both compressed and expanded xylem area vessel elements. The lengths decrease from the innermost (oldest) to the outermost (youngest) secondary xylem. This fact would seem to bear-out the contention of Foster (1949) and others that specialization occurs in the youngest secondary xylem first and develops inward. It was also found that there was a difference in vessel element length between the compressed and expanded xylem areas. Lengths differed from 8 microns to 19 microns for an average difference of 14.5 microns and a variation of 6%. It would appear then that such a variation would be significant and that there were differences in the lengths of the vessel elements of the two xylem areas. Further study of the two areas would be necessary to definitely corroborate the indication of a difference found in this study. If further studies corroborated this work, then the criterion of xylem homogeneity would probably have to be altered.

The diameters of the vessel elements of the expanded xylem area differed from 1-2 microns for an overall average of 50 microns and a variation of 4%. The compressed xylem area vessel elements differed from 2-4 microns for an overall average of 48.5 microns and a variation of 8% between the smallest and the largest diameter.

The largest differences occurred in growth rings 6-10 and 11-15.

Diameters of the expanded xylem area vessel elements were in general larger than those of the compressed xylem area. Both xylem areas exhibited a tendency for the diameters to increase from the oldest to the youngest secondary xylem again supporting the contention of Foster (1949).

With one exception, that of the length-width ratio of growth rings 6-10 of the compressed xylem area, the ratios of the expanded xylem area were smaller than the corresponding ratios from the same growth ring groups of the compressed xylem area. Again, the tendency for specialization to occur first in the outermost or youngest secondary xylem is supported because the ratios in both xylem areas became smaller from the inside towards the outside of the stem.

It would appear that from the data obtained from the compressed and expanded xylem areas there is a difference in the anatomical structure of the xylem. Evidently the xylem is not homogeneous throughout the stem but differs significantly as it matures from the outside towards the inside. The data from this study should serve to strengthen the taxonomic position of this species where it is now placed. In addition, from this study, further anatomical studies of the outermost secondary xylem would be warranted in all "woody" plants to determine whether the suspected xylem homogeneity is a valid belief.

The perforations at the ends of the vessel elements were all found to be simple or porous. There were no indications of scalariform perforations present although there have been reports of foraminite scalariform perforations present in occasional vessel elements (Metcalf and

Chalk, 1950).

Pitting in the lateral walls of the vessel elements varied from elongate to opposite with all transitional types between the two. The most common type of pitting encountered was the alternate type. Alternate pitting is a rather advanced characteristic and indicates a phylogenetic advancement of the tissue of a particular plant.

There was also found in this study the presence of tertiary thickenings in the lateral walls of the vessel elements. These thickenings assumed a spiral shape and are further anatomical evidence for phylogenetic advancement of the tissue.

The end wall angle was found to vary from 45° to 80° . The angle most frequently encountered was approximately 60° . Such an angle would be indicative of a somewhat advanced characteristic.

Vessel elements tended to group together in groups of 2-3 cells as reported by Metcalfe and Chalk (1950). Infrequently, the vessel elements were found to be in groups of 4-6 cells and rarely in groups of as many as 8 cells. Such a tendency for vessel element grouping has not been construed to have any phylogenetic implication. It is, however, an anatomical characteristic of this genus.

Lengths of vessel element "tails" were measured to determine whether or not such measurements were of phylogenetic significance. "Tail" lengths varied from no "tails" present to those 140 microns long. The average length of "tails" in expanded xylem area was 32 microns and that of the compressed xylem area 30 microns. "Tail" lengths in the expanded xylem area were slightly higher than those from the compressed xylem area.

Short "tails" lengths may be indicative of an advanced phylogenetic condition and an absence of any "tail" would appear to indicate a highly advanced characteristic.

From my data on Acer nigrum and that gathered from the literature, indications are that, anatomically, the genus Acer appears to agree with the taxonomic position in which it is placed upon floral anatomy and general morphology. From the anatomical data, the genus would appear to be quite highly advanced phylogenetically.

Platanus occidentalis

The genus Platanus is the only member of the family Platanaceae. The species are monoecious trees. Flowers are unisexual. Staminate and pistillate flowers in separate globular heads which are on pendulous peduncles; staminate flowers several to many in male head, each composed of a calyx cup, 3-7 tridentate and minute petals alternating with the whorl of 3-7 stamens; pistillate flowers hypogynous, perianth present, calyx cupular and 3-5 lobed or of 3-5 distinct sepals, petals generally absent. Ovary is superior, uniloculate, 1-carpelled; 1-2 ovules in the locule. Pistils 5-9 with 1 style. The fruit is a 1-seeded linear quadrangular achene or rarely follicular. Seed with endosperm.

Anatomically, the family is characterized by Solereder (1908) and Metcalfe and Chalk (1950) as follows: (1) Both simple and scalariform perforations with 1-12 bars (Solereder, 1908) or fewer than 20 bars (Metcalfe and Chalk, 1950). (2) Vessel elements solitary and in radial to tangential 2's and 3's. (3) Cell lumen small and (4) Absence of

tertiary thickenings in walls of vessel elements.

The anatomical characteristics of Platanus occidentalis established by this study generally agreed with those of the family. One notable exception was the presence of more than 20 bars comprising a scalariform perforation. There were vessel elements present in both the expanded and compressed xylem areas with 20 bars and others with as high as 30 bars. Other anatomical features found to be present were: (1) vessel element end wall angle of 45° - 60° . (2) lateral wall pitting of vessel element walls generally elongate but with all transitional forms from scalariform to, rarely, opposite. Such additional anatomical characteristics are undoubtedly of phylogenetic importance.

The various anatomical characteristics of Platanus occidentalis found in this study are listed in Table II.

The lengths of the vessel elements in both the expanded and compressed xylem areas were of moderate length in comparison to the other two species of this study. The average length of the vessel elements was approximately 500 microns. In growth ring Group I, (growth rings 1-5), there was very little difference in vessel element length between the expanded and compressed xylem areas. There was a difference of only 7 microns which was a variation of 1.4%. Such a variation would not appear to be significant. Group II, (growth rings 6-10), indicated a difference of 12 microns between the compressed and expanded xylem areas. The variation was 2.4%. Such a variation would appear to be indicative of a significant difference in the vessel element lengths between the two xylem areas. Group III, (growth rings 11-15), indicated a difference of

44 microns between the compressed and expanded xylem area. The variation was 8%. A variation of 8% would appear to be significant and would indicate a marked difference in vessel element length between the two xylem areas.

The average length of vessel elements in the expanded xylem area was 502 microns while that of the compressed xylem area was 481 microns. The difference was 21 microns and a variation of 4%. With this datum as a criterion, it appears that there is a significant difference in vessel element length between the two xylem areas.

The vessel length in the expanded xylem area increased from the oldest (innermost) to the youngest (outermost) secondary xylem. This increase appears to conflict with the contention of Foster (1949) that specialization occurs first in the youngest secondary xylem - specialization here made in reference to vessel element length. Short vessel length is considered a specialization of longer vessel elements. The longer vessel elements would be expected to be in the inner portions (older) of the secondary xylem. The same tendency for an increase in vessel length towards the youngest secondary xylem appeared in the compressed xylem area. Although a slight decrease in length appeared in Group III (growth rings 11-12), it seems that the tendency was the same as that of vessel elements of the expanded xylem area.

A probable explanation for the tendency of vessel elements to increase in length towards the youngest secondary xylem would be rapid cambial activity with a large number of vessel element initials formed combined with slow maturation of the older elements. Perhaps the stem

was not of sufficient age to indicate the specialization which could have occurred. Still another possible explanation would be the use of atypical material which would not be indicative of the true anatomical characteristics of the species.

The results obtained, however, would seem to indicate that there is a difference between vessel element lengths in the compressed and expanded xylem areas.

The widths of the two xylem areas were calculated to have the same average of 69 microns. The widths of the vessel elements of the expanded xylem area varied from 68-70 microns; a variation of 3%. The widths of the vessel elements of the compressed xylem area varied from 67-73 microns; a variation of 8%. From Table II, it can be seen that there was a slight indication of a stabilization of width throughout the secondary xylem. However, in Group I, (growth rings 1-5), there is a variation of 4.3% between vessel element widths of the expanded and compressed xylem areas, a variation of 7% in Group II, (growth rings 6-10), and a variation of 3% in Group III, (growth rings 11-12). These data appear to indicate that a difference might occur between vessel element width of the two xylem areas. Although the averages of the two xylem areas were the same, averages between the same groups of the two different xylem areas were different.

There were no indications, with the possible exception of Group II, (growth rings 6-10), of the compressed xylem area, for the widths of the vessel elements to be greater towards the youngest secondary xylem. The widths of elements from Group I, (growth rings 1-5), of both xylem areas

differed only slightly, if at all, from those of Group III, (growth rings 11-12). Possible explanations of this tendency may be the same as those for the tendency of the vessel elements to be longer towards the youngest xylem.

The length-width ratios of the vessel elements in the three groups of the expanded xylem area increased towards the youngest secondary xylem. Those of the compressed xylem area decreased between Group I, (growth rings 1-5), and Group II, (growth rings 6-10), and increased in Group III, (growth rings 11-12). Here again is conflicting data which appears not to agree with specialization occurring first in the youngest secondary xylem. There were, however, indications that differences did occur between the same growth ring groups of the two different xylem areas. There was a tendency for the ratios in the expanded xylem area to be greater than those of the compressed xylem area suggestive perhaps of the earlier maturation and specialization of the vessel elements in the compressed xylem area.

Vessel element "tail" lengths varied significantly between groups of the same xylem area and between the same groups of the two different areas. The average length of "tails" in the expanded xylem area was 40 microns while that of the compressed xylem area was 48 microns; a variation of 17%. "Tail" lengths increased from 34 microns to 47 microns from the oldest to the youngest secondary xylem in the expanded area but decreased from 53 microns to 43 microns in the compressed xylem area. Variations between the same groups of the two different areas, however, decreased from 36% to 7.5% from the oldest to the youngest secondary

xylem. The data were quite erratic; probably not indicative of the true characteristics present. Many vessel elements had no "tails" and were not included in the count. Perhaps were those vessel elements lacking "tails" included in the count, the significance of the data may have been quite different.

Lateral wall pitting in the vessel elements of both xylem areas varied from scalariform to elongate with occasional alternate and infrequent opposite pitting. The most common pitting type encountered was elongate. This pitting type is indicative of a somewhat primitive characteristic.

The end wall angle of the vessel elements varied from 45° - 60° . The most frequent angle encountered was approximately 45° . Such an end wall angle would seem to indicate a mid-point position between primitive and advanced characteristics.

There were no indications that tertiary thickenings were present in the walls of the vessel elements of either xylem area.

Perforations were found to include both simple (porous) and scalariform types. In the expanded and compressed xylem areas, simple perforations were approximately 70% of the total. In three groups, the simple-scalariform ratios were 2:1, in two groups, 3:1, and in one group, 1.8:1. It would appear that the simple-scalariform ratio was about 2.3:1. The number of bars present in the scalariform perforations varied from 1-30. The average number of bars was 15-18. Encountered quite frequently were vessel elements with both simple and scalariform perforations at opposite ends of the same element. The presence of both perforation types on

different vessel elements, the average number of bars comprising each scalariform perforation, and the occurrence of both simple and scalariform perforations on the same vessel element seem to indicate a condition mid-way in the evolutionary scale based upon anatomical characteristics. Phylogenetically, then, Platanus could possibly be classified as a transitional form between primitive and advanced types.

Rhododendron maximum

The genus Rhododendron is a member of the family Ericaceae whose members are characterized mainly as shrubs and occasionally trees. Flowers bisexual, solitary in axils or in axillary or terminal clusters, racemes, or panicles; calyx small 4-7 lobed, corolla with 4-7, generally 5, petals. Stamens as many as or twice as many as petals borne on a hypogynous disc. Anthers 2-celled. Ovary is superior, 4-10 locular, typically 5; many ovules in each locule. Pistils 1 with usually 1 style. The fruit is a capsule. Seed has fleshy endosperm (Lawrence, 1951).

Anatomically, the family is characterized by Solereder (1908) and Metcalfe and Chalk (1950) as follows: (1) Perforations nearly always scalariform (Solereder, 1908); perforations exclusively scalariform (Metcalfe and Chalk, 1950). (2) Rays uniserriate. (3) Simple and bordered pits in vessel element side walls. (4) Tertiary thickenings spiral in shape and in vessel element "tails" only. (5) Vessel element diameters quite small.

The anatomical characteristics of R. maximum established by this study are listed in Table III. The characteristics listed above were

confirmed in this work. In addition, the following characteristics were observed: (1) Perforations all scalariform. (2) Vessel element end wall angle 30° . (3) Bars in scalariform perforations 5-25 and (4) vessel element side wall pitting generally scalariform and elongate.

The average length of the vessel elements from the expanded xylem area was 379 microns and that from the compressed xylem area was 376 microns. The difference in length was 3 microns and a variation of less than 1%. Calculations from the overall averages would not indicate a significant difference in the vessel element length of the two xylem areas. The vessel elements in both xylem areas exhibited a tendency to increase in length from Group I, (growth rings 1-5), to Group III, (growth rings 11-15), but decreased in length somewhat sharply in Group IV, (growth rings 16-20). The difference in vessel element length between the same groups of the two different xylem areas did not vary greatly. The difference in Group I was 10 microns for a variation of 2.6%, Group II difference was 1 micron for a variation of less than 1%, Group III difference was 5 microns for a variation of 1.3%, and Group IV difference was 18 microns for a variation of 5%. The difference and variation in Group IV appears to be significant although a sudden change does not seem likely. However, a possible explanation of the sudden change may be the rapid increase in maturation rate with the subsequent expression of specialization. There does not appear to be, however, any data present which would indicate conclusively that a significant difference occurs between the vessel element length of the two xylem areas. An apparent explanation for the similarity in structure of the two xylem areas would

be the primitiveness of the species. Evidently, the species has not developed sufficiently to express specialization if there is a difference in the two areas.

The difference in average vessel element width between the two xylem areas was 0.4 micron for a variation of 1.2%. Such an average difference would not appear to be significant. The xylem elements in the expanded xylem area exhibited a tendency to increase in width from Group I to Group III and stabilize thereafter. The elements in the compressed xylem area exhibited a similar tendency to increase in width from Group I to Group III but decreased somewhat in width in Group IV. It would appear that there is present a tendency for increase in width from the oldest to the youngest secondary xylem.

The differences between the same groups of the two different xylem areas were variable - the largest variation being in the older secondary xylem. The difference in Group I was 2.4 microns for a variation of 5%, Group II difference was 1.8 microns for a variation of 5%, Group III difference was 0.2 micron for a variation of less than 1%, and Group IV difference was 1 micron for a variation of 3%. Significant differences appear to occur in Groups I, II, and possibly in Group IV. There was no significant difference in Group III.

Apparently, there is a tendency for the vessel element width to increase from the oldest to the youngest secondary xylem. In addition, significant differences appear to occur between the same groups of the two different xylem areas.

The length-width ratios of both the expanded and compressed xylem

area vessel elements decreased from the oldest to the youngest secondary xylem. This tendency would support the contention of Cox (1948) that length-width ratios are reduced in specialized areas. It would also support the contention of Foster (1949) that specialization occurs first in the youngest secondary xylem (a low length-width ratio being considered an advanced characteristic).

There did not appear to be any significant differences between the ratios of the same groups of the two different xylem areas. Apparently then there is no difference in the anatomical structure of the vessel elements comprising the two secondary xylem areas.

The average vessel element "tail" lengths of the two xylem areas differed by 1.4 microns for a variation of 3.6%. Large differences occurred between Groups I and II of the two xylem areas. However, the "tail" lengths of Group III were identical and those of Group IV differed by 1.4 microns. The difference in Group IV was a significant decrease from those differences in Groups I and II. It would not appear that significant differences occur in the outer portions of the secondary xylem, (the area where specialization is first expressed), but that significant differences do occur in the inner portions.

The end wall angle of the vessel elements was found to be 30° . There was a slight variation from this angle in two groups of the compressed xylem area but they were not of a magnitude to assume that the average angle of 30° is indicative of a primitive condition.

Perforations at the ends of the vessel elements were found to be entirely scalariform. The bars comprising the scalariform perforations

varied in number from 5-26 with the average being 12-15 bars. Scalariform perforations are indicative of a primitive condition and the number of bars comprising each perforation would support the condition of primitiveness.

Lateral wall pitting was found to vary from scalariform to elongate with an occasional appearance of opposite. There did not appear to be a definite type always encountered. The presence of scalariform and elongate types appeared to be of an equal frequency. Both scalariform and elongate pitting are primitive anatomical characteristics - elongate pitting being derived from scalariform pitting.

Tertiary thickenings were encountered in nearly every vessel element observed. Furthermore, the thickenings were spiral in shape and occurred only in the "tails" of the vessel elements. Tertiary thickenings are considered advanced phylogenetically but since they occurred only in the "tail" section of vessel elements, specialization evidently has not advanced too far. However, the presence of these thickenings indicates that specialization of the vessel elements has begun and has progressed beyond the primitive stage.

Anatomically, R. maximum may be classified as a species which has not become specialized far beyond the primitive state. This classification agrees with the accepted position of the species.

Conclusions

1. Anatomical characteristics of the secondary xylem from a stem of Acer nigrum are phylogenetically advanced and corroborate the accepted taxonomic position of the species. Specialization appears to occur first in the youngest secondary xylem and develop inward towards the oldest tissue. There appears to be a significant difference between the anatomical characteristics of the vessel elements comprising the expanded and compressed secondary xylem areas.
2. Anatomical characteristics of the secondary xylem from a stem of Platanus occidentalis are advanced beyond the primitive stage but are not considered highly advanced. The species appears to be in a transitional stage between primitive and advanced types. Specialization seemed to occur from the innermost (older) to the outermost (younger) secondary xylem - a contradiction to the generally accepted theory of Foster (1949). There were indications of a difference between anatomical characteristics of the vessel elements of the two secondary xylem areas.
3. Anatomical characteristics of the secondary xylem of Rhododendron maximum are phylogenetically primitive and corroborate the accepted taxonomic position of the species. Specialization appears to occur in the youngest secondary xylem and develop inward towards the oldest secondary xylem. There did not appear to be significant differences in the anatomical characteristics of the vessel elements of the two secondary xylem areas.
4. Anatomically, Rhododendron maximum may be considered primitive,

Platanus occidentalis advanced over R. maximum, and Acer nigrum
advanced over both R. maximum and P. occidentalis.

Summary

1. The literature was reviewed for information regarding anatomical studies of secondary xylem - particularly information pertaining to Acer nigrum, Platanus occidentalis, and Rhododendron maximum.
2. Tissue used in the experiment was obtained from the secondary xylem area of well-developed limbs of all three species.
3. Inspection of the secondary xylem of all three species indicated variations in the width of growth rings. Growth rings were small or compressed on one side of the limb and large or expanded on the opposite side of the limb. Material from both growth ring areas was obtained and was studied separately.
4. Tissue maceration was accomplished using Jeffrey's technique (modified). Macerated material was handled according to the technique of Cox (1948).
5. Microscopic investigation of the expanded and compressed xylem areas of Acer nigrum indicated a difference in anatomical structure of the vessel elements of the two areas. Anatomical differences were expressed mainly by the lengths, widths, and length-width ratios of the vessel elements comprising the xylem tissue.
6. Microscopic investigation of the expanded and compressed xylem areas of Platanus occidentalis indicated a possible difference in the anatomical structure of the vessel elements of the two areas. Specialization of tissue appeared to occur from the oldest to the youngest xylem - seemingly contradictory data to the accepted belief in which specialization occurs from the youngest to the oldest xylem.

Anatomical characteristics were expressed in the same manner as those of A. nigrum.

7. Microscopic investigation of the expanded and compressed xylem areas of Rhododendron maximum did not indicate a significant difference in the anatomical structure of the vessel elements comprising the two areas. The vessel element lengths of both areas, however, indicated a tendency to become markedly shorter in the outermost (youngest) secondary xylem.

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Vita

Robert Stanley Leisner was born in Washington, D. C. on August 9, 1927 - the second son of Evelyn V. and John P. Leisner. He attended public school in Alexandria, Virginia, 1933-1940, and was graduated from Mount Vernon High School in 1944. In 1949, he was honorably discharged from the United States Navy after service of four years. He was awarded a B.S. in General Science from Virginia Polytechnic Institute, Blacksburg, Virginia in June, 1953. During candidacy for an M.S. in Botany, he held a Teaching Assistantship at Virginia Polytechnic Institute, 1953-1955.

He is a member of College leaders - 1953 and a member of the Civilian Student Body Senate, 1954-1955. At the present time, he is unmarried.

Robert S. Leisner

TABLE I

ANATOMICAL CHARACTERISTICS OF THE SECONDARY XYLEM FROM THE STEM OF ACER NIGRUM

Vessel Elements	EXPANDED XYLEM AREA				COMPRESSED XYLEM AREA			
	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-15	Growth Rings 16-20	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-15	Growth Rings 16-20
Maximum Length	330 μ	359 μ	330 μ	337 μ	400 μ	380 μ	380 μ	359 μ
Minimum Length	180 μ	187 μ	150 μ	144 μ	180 μ	186 μ	194 μ	180 μ
Average Length	266 μ	267 μ	259 μ	244 μ	285 μ	275 μ	274 μ	260 μ
Maximum Width	72 μ	72 μ	64.5 μ	79 μ	72 μ	65 μ	72 μ	86 μ
Minimum Width	21 μ	29 μ	28.7 μ	29 μ	29 μ	29 μ	29 μ	29 μ
Average Width	49 μ	50 μ	50 μ	51 μ	50 μ	46 μ	48 μ	50 μ
Maximum "Tail" Length	86 μ	72 μ	71 μ	143 μ	100 μ	79 μ	86 μ	79 μ
Minimum "Tail" Length	14 μ	14.4 μ	14 μ	14 μ	14 μ	14 μ	14 μ	14 μ
Average "Tail" Length	34 μ	31 μ	29 μ	33 μ	29 μ	32 μ	30 μ	29 μ
Length-Width Ratio	5.4:1	5.3:1	5.1:1	5.0:1	5.7:1	6.0:1	5.7:1	5.2:1
End Wall Angle	45° - 80°	45° - 80°	45° - 75°	60°	45° - 80°	45° - 80°	45° - 80°	45° - 80°
Perforations	Simple	100%	100%	100%	100%	100%	100%	100%
	Scalariform	—	—	—	—	—	—	—
No. of Scalariform Bars	None	None	None	None	None	None	None	None
Lateral Wall Pitting	Alter-nate	Alter-nate	Alter-nate	Alter-nate	Alter-nate	Alternate & Elongate	Alternate & Opposite	Alternate & Opposite
Tertiary Thickening	Present	Present	Present	Present	Present	Present	Present	Present

TABLE II

ANATOMICAL CHARACTERISTICS OF THE SECONDARY XYLEM FROM THE STEM OF PLATANUS OCCIDENTALIS

Vessel Elements	EXPANDED XYLEM AREA			COMPRESSED XYLEM AREA		
	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-12	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-12
Maximum Length	704 μ	796 μ	760 μ	675 μ	645 μ	693 μ
Minimum Length	323 μ	316 μ	280 μ	316 μ	285 μ	302 μ
Average Length	481 μ	500 μ	524 μ	474 μ	488 μ	480 μ
Maximum Width	100 μ	115 μ	100 μ	93 μ	73 μ	86 μ
Minimum Width	36 μ	36 μ	43 μ	43 μ	43 μ	36 μ
Average Width	70 μ	68 μ	69 μ	67 μ	73 μ	67 μ
Maximum "Tail" Length	122 μ	129 μ	122 μ	144 μ	122 μ	101 μ
Minimum "Tail" Length	14 μ	14 μ	14 μ	14 μ	14 μ	14 μ
Average "Tail" Length	34 μ	40 μ	47 μ	53 μ	48 μ	43 μ
Length-Width Ratio	7.0:1	7.4:1	7.5:1	7.1:1	6.6:1	7.2:1
End Wall Angle	45° - 60°	45° - 60°	45° - 60°	45° - 60°	45° - 60°	45° - 60°
Perforations	Simple	68%	66%	74%	66%	74%
	Scalariform	32%	34%	26%	34%	26%
No. of Scalariform Bars	2-30	1-25	2-30	1-22	2-20	2-25
Lateral Wall Pitting	Elongate	Elongate	Elongate	Elongate	Elongate	Elongate
Tertiary Thickening	Absent	Absent	Absent	Absent	Absent	Absent

TABLE III

ANATOMICAL CHARACTERISTICS OF THE SECONDARY XYLEM FROM THE STEM OF RHODODENDRON MAXIMUM

Vessel Elements	EXPANDED XYLEM AREA				COMPRESSED XYLEM AREA			
	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-15	Growth Rings 16-20	Growth Rings 1-5	Growth Rings 6-10	Growth Rings 11-15	Growth Rings 16-20
Maximum Length	595 μ	646 μ	590 μ	618 μ	617 μ	682 μ	675 μ	603 μ
Minimum Length	158 μ	180 μ	144 μ	172 μ	172 μ	158 μ	180 μ	172 μ
Average Length	370 μ	382 μ	391 μ	373 μ	380 μ	383 μ	386 μ	355 μ
Maximum Width	41 μ	48 μ	50 μ	51.4 μ	43.0 μ	48 μ	46 μ	56 μ
Minimum Width	16.6 μ	21.5 μ	16.6 μ	21.5 μ	18.0 μ	18.3 μ	21.7 μ	17.0 μ
Average Width	27.6 μ	34.5 μ	35 μ	34.8 μ	29 μ	32.7 μ	34.8 μ	33.8 μ
Maximum "Tail" Length	158 μ	108 μ	129 μ	115 μ	94 μ	115 μ	144 μ	115 μ
Minimum "Tail" Length	14 μ	14 μ	14 μ	14 μ	14 μ	14 μ	14 μ	14 μ
Average "Tail" Length	38.7 μ	34.5 μ	40 μ	36 μ	34.3 μ	43 μ	40 μ	37.4 μ
Length-Width Ratio	13.4:1	11.1:1	11.2:1	10.7:1	13.2:1	11.7:1	11.1:1	10.5:1
End Wall Angle	30°	30°	30°	30°	30°	30°	20° - 30°	30° - 45°
Perforations	Simple	—	—	—	—	—	—	—
	Scalariform	100%	100%	100%	100%	100%	100%	100%
No. of Scalariform Bars	5-25	8-21	5-26	5-18	5-20	5-20	5-20	4-18
Lateral Wall Pitting	Scalariform	Elongate Opposite	Scalariform & Opposite	Scalariform & Elongate	Scalariform	Scalariform & Elongate	Scalariform & Elongate	Scalariform & Elongate
Tertiary Thickening	In "Tails"	In "Tails"	In "Tails"	In "Tails"	In "Tails"	In "Tails"	In "Tails"	In "Tails"