LEAF ANATOMY AND ONTOGENY IN ARGYROXIPHIIUM AND WILKESIA (COMPOSITAE)

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The four species of the genus Argyroxiphium (commonly known as the "silverswords") and the related monotypic genus Wilkesia are narrowly-restricted Hawaiian endemics. They have been classified with the tarweeds (Heliantheae, subtribe Maidiinae) by Hoffmann (1890), although Keck (1936) has expressed a dissenting opinion. As in few other genera, remarkable specializations of habit and habitat make them intriguing objects for anatomical study. Moreover, anatomical information should aid in clarifying their systematic relationships, as the writer hopes to demonstrate in ensuing researches.

Argyroxyphium sandwichense DC., well known as the "silversword" because of the shining felty indument on its leaves, occurs in dry volcanic craters on the islands of Maui and Hawaii. It is monocarpic and has the habit and size of an acaulescent Yucca. The closely related A. caliginii Forbes, from an extremely moist mountain top on West Maui, is a smaller, low branched rosette plant, not monocarpic. Also in the same location is A. grayanum (Hillebr.) Degener, which is somewhat shrubbier; its leaves, which are flat and wide, lack the prominent covering of hairs characteristic of the two preceding species. Similar in leaf indument to A. grayanum is A. virescens Hillebrand, sometimes known as the "greensword." Argyroxiphium virescens, like A. sandwichense, is monocarpic and has thick, almost quadrangular leaves (fig. 16). It occurs in moderately moist habitats on the slopes of Haleakala, Maui. Wilkesia gymnoxiphium Gray is also monocarpic, although its habit is arrestingly different from that of any other Compositae. Its thin, lanceolate leaves are borne in verticils, with the leaf bases united into a common tubular sheath at each node; the stems are long and pole-like. Wilkesia grows in relatively dry forest areas on Kauai. For photographs and drawings showing the habit and gross morphology of these genera, the reader is referred to Keck (1936), Degener (1946) and Carlquist (1955).

Materials and Methods.—Portions of field-collected specimens of all species (except A. virescens, which was available as herbarium material) were preserved in Carnoy’s fluid. This fixative, although inadvisable for general work, was excellent here, because its anhydrous nature permitted the preservation of intercellular deposits of pectic compounds. Supplementary material from a cultivated plant of A. sandwichense (courtesy of Dr. H. L. Mason) and Wilkesia (cultivated by the University of California Botanical Garden from the writer’s seed collections) was preserved in formalin-propionic-alcohol (Johansen, 1940). Leaves of A. virescens (Forbes 1251M, UC) were treated with 2.5 per cent aqueous sodium hydroxide to restore their shape and to remove discoloration. Johansen’s tertiary butyl alcohol series, beginning at 85 per cent aqueous sodium hydroxide to restore their shape and to remove discoloration. Johansen’s tertiary butyl alcohol series, beginning at 85 per cent alcohol, was used for dehydration, and material was infiltrated and embedded in paraffin according to the usual techniques. Paraffin ribbons were mounted with minimal exposure to aqueous solutions, and an entirely anhydrous staining series was used. Safranin and fast green dissolved in

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absolute ethyl alcohol were employed. For identification of pectic compounds, as well as for a permanent stain on sections, Ruthenium red dissolved in 70 per cent ethyl alcohol was used. The pectic nature of intercellular accumulations was also established by their solubility in warm 0.1 per cent ammonium oxalate; water alone, however, can gradually leach out these compounds. On this account, and because of the dense trichomes and fibers in some species, leaves of *Argyroxyphium* proved exceptionally difficult microtechnical objects.

Herbarium specimens documenting the writer's collections have been deposited in the University of California Herbarium, Berkeley.

*Argyroxyphium*: leaf anatomy.—As shown in the photograph (fig. 6) or diagram (fig. 16) of leaf transections of *Argyroxyphium*, two anomalous features require explanation. First is the presence of loosely-arranged mesophyll parenchyma, the intercellular spaces in which are partly (fig. 6) or completely (fig. 11) filled with pectic compounds. This pectic material, although apparently not present in juvenile leaves of *A. sandwichense*, is characteristic of adult leaves of all of the species, and is present in fresh material in jelly-like form. Such pectic materials are present in channels of mesophyll which run the length of the leaf between vein sheath extensions. The term “pectic channels” is applied here to this type of distribution.

The second noteworthy feature is the presence and orientation of three sets of bundles in the leaf. As indicated in both fig. 6 and fig. 16, there are three sets of bundles, designated here as upper, middle, and lower sets. The lower set of bundles is inverted in orientation, whereas upper and middle sets are normal.

These anomalous features are best explained by study of the leaf ontogeny. For this purpose, preparations of *A. caliginii* proved most suitable, although similar features were observed in *A. sandwichense*. The shoot apex (fig. 1), which is rather flat, shows two tunica layers. A short distance below the central mother cell zone, cells derived from rib meristem mature into lacunate pith parenchyma. This short region of meristematic activity is associated with the rosette habit of the plant. Observations on cell lineages in tips of young leaf primordia indicate that marginal and submarginal initials are present. The submarginal initials give rise to a hypodermal layer on both surfaces of the leaf; they also produce actively dividing cells which soon mature into the numerous layers of the massive mesophyll. In relatively young primordia, periclinal divisions can be seen in the hypodermal layer of both surfaces. Such divisions in the adaxial surface are relatively few, and only three layers of palisade parenchyma (fig. 7) arise from this hypodermal layer. On the abaxial surface, however, periclinal divisions begin early (fig. 3) and are quite numerous, producing files of cells. Active divisions occur to either side of the midvein, although not beneath it (fig. 4); this is associated with the fact that only two pectic channels are present in the mature leaf. The actively dividing cells on the abaxial surface are collectively termed the “abaxial meristem” here. Its products can be delimited by the fact that adjacent to the inner surface of cells derived from the abaxial meristem are dark-staining intercellular pectic deposits (fig. 4). As may be seen in longitudinal section (fig. 5), the abaxial meristem is only weakly developed toward the base of the primordium (at right). Its products include not merely palisade parenchyma, but also the procambium of the lower set of bundles, and the lower portions of the pectic channels (fig. 2, below white lines). At the same time, other portions of the primordium within the future adaxial palisade produce two series of procambial strands and most of the mesophyll parenchyma. As fig. 4 shows, the middle bundles of the leaf develop first, in sequence from midvein to margins. Procambium originating the upper set of bundles and procambium connecting the middle and upper bundles (fig. 2, above) develop next. Simultaneously with maturation of vascular elements, a pair of secretory canals, sometimes only one, differentiates near the protoxylem of the larger veins (fig. 4, 7).

The mature condition is shown in fig. 6 for *A. sandwichense*. This photograph illustrates a number of features which merit mention: (1) the midvein is provided with a bundle-sheath extension connected with both epidermal layers, and bundle sheaths are developed on smaller veins. (2) Along the lower surface, a lower set of inverted bundles is present. (3) Between the palisade parenchyma and the pectic channels there are compact, isodiametric parenchyma cells which lack chloroplasts and are similar to those of the vein-sheath extension. The vascular bundles and fiber strands are embedded in this parenchyma. Although some of these strands of fibers are associated with veins, others appear to lack conducting tissues. Some bundles may appear to be “isolated” within the pectic channels (fig. 6, upper right). These originate from the middle series, and are like the midvein except that they possess no sheath extensions. Leaves on some plants of *A. caliginii* and *A. sandwichense* are flatter, and show a slightly different structure. In these, veins of the middle set other

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Fig. 6-9.—Fig. 6. *Argyroxyphium sandwichense*, Carlquist H28, portions of transection of mature leaf.—Fig. 7. Adaxial face above. ×96.—Fig. 7-8. *A. caliginii*, Carlquist H28, portions of transection of mature leaf.—Fig. 7. Portion of adaxial surface. ×180.—Fig. 8. Portion of margin. ×245.—Fig. 9. *A. sandwichense*, Carlquist H19, cells from longitudinal section of pith. ×400.
than the midvein develop complete bundle-sheath extensions, so that several, not just two, pectic channels are present.

In summary, all but the basal portion of adult leaves of *A. caliginii* and *A. sandwichense* contain three series of veins. Only the middle set is present in the leaf base (fig. 17) or in juvenile leaves. The middle set of veins forms anastomoses, as does the upper set, and veins connecting these two sets occur. The veins of the lower set, which are inverted, originate from the abaxial meristem. Because of this, they connect with the other veins only along the leaf margins, where lower veins join with the marginal veins of the middle set; and at the leaf tip, where all veins join in an amphiphloic plexus. Since the abaxial meristem is absent from the leaf base, there are no lower veins in this region. Although the lower veins do not connect with the other sets except at the margins of the leaf, they frequently anastomose with each other. The upper set of bundles is not present in the leaf base because they branch from the middle set at points above the base.

**ARGYROXIPHIUM: COMPARISON OF SPECIES.**—On account of the wide disparity in habitat between the similar pair of species, *A. sandwichense* and *A. caliginii*, the writer was interested in noting anatomical differences in their leaves. As comparison of fig. 6 and 7 shows, *A. caliginii* possesses paired secretory canals along the major veins, whereas they are totally lacking in *A. sandwichense*. These canals contain an oleoresinous substance and are schizolygenous, since some cells are destroyed in their formation, although development is marked predominantly by separation of the cells. Although secretory canals flanking veins which continue into the stem as leaf traces also continue downward into the stem, secretory canals adjacent to the other veins terminate freely in the leaf base. *Argyroxyphium sandwichense* has a dense coating of thick-walled hairs on its leaves; epidermal cells have a prominent cuticle. These trichomes are exclusively uniseriate and non-glandular. Biseriate glandular hairs of the type shown in fig. 11, however, occur on the leaf base and on juvenile leaves, intermixed with the uniseriate type. The uniseriate trichomes
Fig. 1–5. *Argyroxyphium caliginii*, Carlquist H28.—Fig. 1. Longitudinal section of shoot apex. ×204.—Fig. 2. Longitudinal section of leaf primordium, showing a portion more distal from the leaf base than fig. 5. Layers below white lines on edge of photograph derived from abaxial meristem. ×195.—Fig. 3. Portion of transection of young leaf primordium, showing periclinal divisions in the abaxial hypodermal layer. ×410.—Fig. 4. Transection of older primordium, showing development of abaxial meristem (below dark line paralleling lower face). ×208.—Fig. 5. Longitudinal section of leaf primordium near base, showing abaxial meristem; leaf base at right. ×270.
are upwardly appressed, and flattened in their distal portions. This flattening makes them lens-shaped in transection, and may account for the unusual sheen of the leaf. *Argyroxyphium caliginii*, on the contrary, has thin-walled epidermal cells and trichomes. The trichomes are upwardly-appressed but not flattened. They are less numerous than those of *A. sandwichense*. Another difference between the two species is indicated in fig. 8. Massive hydathodes are present along the margins of the leaf in *A. caliginii*, whereas they are lacking in *A. sandwichense*.

The difference between these species may in part be interpreted as having ecological significance. The denser coating of thick-walled hairs, the conspicuous cuticle, and the absence of hydathodes in *A. sandwichense* may be correlated with its sunny dry habitat, and the contrasting characters of *A. caliginii* may correspond with the bog-like habitat in which it occurs.

*Argyroxyphium virescens* (fig. 16) and *A. grayanum* (fig. 10) are similar in the sparsity of trichomes on their leaves. This results in a greenish color in contrast to the silvery gray of *A. sandwichense*. In both *A. grayanum* and *A. virescens*, a marked cuticular relief, absent in *A. caliginii* and *A. sandwichense*, is present. Like the latter species, *A. grayanum* and *A. virescens* have pectic channels and an inverted series of lower bundles. There is a difference between these species, however, in respect to the distribution of pectic channels and vein-sheath extensions. In the flat leaves of *A. grayanum*, numerous veins with sheath extensions run parallel to the midvein, with pectic channels intervening between them. There is usually only a single layer of mesophyll parenchyma separating the pectic channels from the palisade parenchyma. These conditions are in contrast to those found in *A. virescens* (fig. 16), in which leaves are narrow and thick. Only two to five pectic channels run parallel to the midvein. Moreover, vein-sheath extensions are wide, and are continuous with broad bands of similar parenchyma which underlie the palisade parenchyma on both leaf surfaces. The underlying parenchyma contains additional pectic channels, a peculiarity found only in *A. virescens*. Such additional channels are probably the product of an abaxial meristem, and because of the abundance of the compact parenchyma, are usually not continuous with the pectic channels in the central portion of the leaf. In all other species (viz., *A. caliginii* fig. 2), the portion of the pectic channels produced by the abaxial meristem merges imperceptibly with that produced by the remainder of the leaf. *Argyroxyphium virescens* typically has two layers of palisade cells on both leaf surfaces, whereas the other species have two on the abaxial face and three on the adaxial. Neither *A. virescens* nor *A. grayanum* has secretory canals in the leaf.

**INFLORESCENCE BRACTS.**—Despite distinctive patterns of leaf anatomy in the four species, their infructescence bracts tend to have a similar structure, shown for *A. sandwichense* in fig. 11. Particularly noteworthy is the presence of numerous biseriate glandular hairs, which are usually absent from leaves of the vegetative plant. These trichomes (fig. 11, lower right) characteristically have multicellular glandular heads, the result of subdivision in the terminal portion of this biseriately structured leaf. A few uniseriate non-glandular hairs are also present. Like vegetative leaves, bracts have photosynthetic palisade parenchyma, usually two cell layers on each surface. Bracts are much flatter and wider than vegetative leaves, and therefore have much the same distribution of vein extensions and pectic channels as leaves of *A. grayanum*. Pectic channels are reduced in size as compared to those of vegetative leaves, so that in this respect the infructescence bracts of *A. sandwichense* are almost identical to the leaves of *A. grayanum*, rather than to leaves of *A. sandwichense*, which show a limited number of broad pectic channels (fig. 6). The middle series of bundles is well developed, and a few upper and lower bundles, the latter inverted in orientation, also occur. The epidermis is not heavily cutinized, although the bract in the fresh condition is coated with the oleoresinous secretion of the numerous glandular hairs.

**WILKESIA: ANATOMY OF FOLLAR APPENDAGES.**—Because *Wilkesia* is markedly different in anatomy from the species of *Argyroxyphium*, it is best treated separately. The shoot apex (fig. 12) is more convex. Sections of several apices indicated that despite plastochronic intervals associated with the verticillate phyllotaxy, the apical meristem has the same shape at all times. Lateral bud primordia, however, have a flat apical meristem, and rarely develop into lateral shoots. Two or three layers of tunica are present, and as in *Argyroxyphium*, a central mother cell zone can be defined. Corresponding to the longer internodes, the zone of rib meristem is of greater vertical extent than in *Argyroxyphium*.

A segment of the free portion of the lamina is shown in fig. 14. In contrast to the leaf of *Argyroxyphium*, *Wilkesia* has a virtually "normal" anatomy. Both biseriate glandular and uniseriate non-glandular trichomes are present. Uniseriate trichomes are more abundant on the upper surface, whereas the glandular trichomes are mostly on the lower surface. The majority of the latter are degenerate and non-functional in the mature leaf. Within the leaf, only a single series of bundles is present. Larger veins are provided with sheath extensions, although only bundle sheaths occur on smaller veins. As in *Argyroxyphium*, prominent fiber strands occur on both faces of a vascular bundle. Such fibers form the adaxial portion of the sheath extension above many larger veins. Although fibers may not be closely associated with the conducting elements, they are always formed in close relation to the bundle, and never are separate from the vein, as
Fig. 12-15. Wilkesia gymnoxiphium, Carlquist H10.—Fig. 12. Longitudinal section of shoot apex. X233.—Fig. 13. Transection of inflorescence bract, adaxial face above. X147.—Fig. 14. Transection of lamina, adaxial face above. X154.—Fig. 15. Transection of sheathing leaf base, adaxial face at left. X150.

they often are in Argyroxiphium. Independent fiber strands, however, do occur at leaf margins. No secretory canals occur in the leaf of Wilkesia. All mesophyll parenchyma, with the exception of the bundle sheaths, is chlorenchyma, and no secretion of pectic materials into intercellular spaces occurs. Two sharply-defined layers of palisade parenchyma are ordinarily present. In respect to
overall venation, leaves of *Wilkesia* show a more strictly longitudinal orientation of veins, with fewer cross-connections than in *Argyroxyphium*.

The basal portion of the leaf of *Wilkesia* (fig. 15), which is united with others at the node, lacks prominent chlorenchyma, although some cells contain a few chloroplasts. The mesophyll consists of relatively compact isodiametric parenchyma cells, although a few spongy cells are present. Just as there is no differentiation between parenchyma surrounding bundles and other mesophyll parenchyma, fibers, which occur more abundantly at the phloem poles than at the xylem poles, tend to intergrade with the parenchyma cells near the bundles. Occasional biseriate glandular hairs (the capitate portion not further subdivided) are present on the abaxial surface of the sheath.

Inflorescence bracts of *Wilkesia* (fig. 13) are quite different from the foliage leaves. Biseriate glandular hairs with short stalks and multicellular heads are present on both surfaces, although more occur on the abaxial surface. Two layers of palisade parenchyma are present on the abaxial surface of the bract; and the remaining spongy parenchyma of the mesophyll contains chloroplasts. Although bundles are provided with parenchyma cells differentiated as a bundle sheath, no sheath extensions are present. No fibers occur in connection with the veins or elsewhere in the bract.

**NODAL ANATOMY.**—Because of the difference in phylloxy between the spiral rosette of *Argyroxyphium* and the verticillate arrangement in *Wilkesia*, the anatomy of the nodes is of interest. Figure 17 represents the pattern of leaf traces observed in *A. sandwichense*. The node is pentalacunar, with three unbranched central traces. Each of the marginal traces, however, branches shortly above its departure from the vascular cylinder of the stem. Half of the veins derived from a lateral trace enter the margin of one leaf, the other half enter the margin of an adjacent leaf. The halves subdivide into numerous veins in the leaf base. This condition obtains also in *A. grayanum*. In *A. caliginii*, however, fewer veins arise from each half of the lateral veins. In all species of *Argyroxyphium*, adjacent margins of leaf bases are connate for a short distance at the base. Mention should be made of the fact that an occasional specimen of *A. grayanum* has verticillate leaves. It is significant that in a seedling of *A. sandwichense*, the writer observed juvenile leaves arranged in pairs, contrary to the supposition of Keck (1936) that they are spiral even at this stage. This observation may help to explain Hildebrand's (1898) statement that juvenile plants of *A. grayanum* have verticillate leaves; his description of this species erroneously assumes that this condition is also typically present in the adult plant. Most mature specimens, however, show only a dense rosette of spirally-arranged leaves. Thus, a transition from opposite to alternate occurs, as in *Helianthus* (Esau, 1945). The fact that occasional specimens of *A. grayanum* have verticillate leaves suggests the possibility that a verticill composed of multilacunar nodes may occur, a condition which has not yet been observed in dicots according to Dr. I. W. Bailey (personal communication). The opposite leaves in the seedling of *A. sandwichense* are associated with trilacunar nodes, and lateral veins of adjacent margins fuse before entering the vascular cylinder.

As shown in fig. 18, *Wilkesia* has trilacunar nodes. The nodal structure is quite different from that of *Argyroxyphium* in respects other than number of gaps, however. The midvein of the leaf is unbranched throughout its length. The two lateral traces typically branch twice at the base of the sheath. Farther up, other branches diverge toward the margins, forming the entire vascular system of the leaf except for the midvein. In contrast to the situation in *Argyroxyphium*, lateral traces of adjacent leaves of *Wilkesia* originate from independent leaf gaps. Of additional interest is the fact that the midvein of a given leaf lies directly between the laterals of a pair of leaves at the node below.

**DISCUSSION.**—The occurrence of pectic channels in leaves of *Argyroxyphium* appears to be an extreme development of the phenomenon designated as "pectic warts" by the writer (1956). That this is indeed the nature of these channels is suggested by the fact that pith of *Argyroxyphium sandwichense* (fig. 9), although not filled with pectic materials, does possess pectic warts. It is curious that these compounds are so much more abundant in the leaves. Although the secretion of pectic compounds in other plants may have been overlooked, owing to microtechnical methods which tend to destroy them, the mucilaginous type of pectic warts reported in peduncles of *Narcissus pseudo-narcissus* (Mangin, 1893) may be a similar phenomenon.

On account of the subterete shape of the leaf in some species of *Argyroxyphium*, and the isolateral structure in all species, it may be tempting to regard the leaf as phylogenetically "inrolled" compared with the normal leaf of *Wilkesia*. That this is not the case is suggested by ontogeny. Early stages, containing only the middle series of bundles, comparable to the single series in leaves of *Wilkesia*, indicated the basic normal condition. Later development of procambium connects with, and forms, the upper set of bundles toward the adaxial surface. The central set of bundles is not located in close proximity to the palisade cells on each surface. It seems logical that the two additional sets of bundles are formed near this chlorenchyma, if the considerations of Haberlandt (1914) regarding the close relationship between vascular and photosynthetic tissue are valid. Although origin of the lower set of bundles from a special meristem results in their connection only with the marginal bundles of the middle set, this connection may well be sufficient for their functioning.
The inverse orientation of the lower set of bundles is probably analogous to the action of the well-known adaxial meristem (Foster, 1936), which adds bundles, inverted in arrangement, to the adaxial face of leaves, particularly in their petiolar regions. Slides of young leaves of Agave prepared by Mr. Howard Arnott (unpublished) show that such meristematic action, producing vascular bundles, is present on both faces of the leaf in that genus. The origin of the upper set of bundles in the Argyroxiphium leaf in a manner different from the abaxial set is curious, however.

The extremely disparate habitats occupied by different species of Argyroxiphium can be related to anatomical differences only to a limited extent. Ecological interpretation of the complicated venation and of the pectic channels, however, cannot be offered readily. It may be that accumulation of pectic compounds aids survival in a xeric habitat, and is quite neutral in a mesic situation. We have no direct evidence, however, that Argyroxiphium was primitively adapted primarily to dry habitats.

On the basis of gross morphology, Keck (1936) assumed that Wilkesia was closely related to, and derived from, Argyroxiphium grayanum. Although evidence is required from all parts of the plant before drawing phylogenetic conclusions, the writer does not believe that the highly specialized type of leaf structure found in Argyroxiphium could give rise to the normal conformation of Wilkesia leaves. Within the genus Argyroxiphium, the leaf anatomy of A. grayanum seems less specialized in some respects, although the presence of secretory canals (found only in A. caliginii) has often been interpreted as primitive in Compositae (for a discussion, see Carlquist, 1957).

Although other types of data are desirable, the features of foliar and nodal anatomy suggest the maintenance of Argyroxiphium and Wilkesia as independent genera, rather than their union into a single genus (e.g., Keck, 1936).

**SUMMARY**

All species of the genus Argyroxiphium have thick leaves in which three sets of bundles are present. The middle set is interpreted as equivalent to the bundles of a normal leaf, a view suggested by their ontogeny and downward extension as leaf traces. An upper set, which is in continuity with the middle set, is formed later near the adaxial surface. A lower set of bundles, contemporary in origin with the upper set, arises through the action of a special meristem, termed the "abaxial meristem." These bundles are connected only with marginal bundles of the middle set, and are inverted in orientation, like those produced by adaxial meristems of other plants. An additional anomalous feature is the presence in the mesophyll of channels of loosely-arranged parenchyma which run the length of the leaf and have intercellular spaces filled with pectic compounds. This type of pectic...
secretion is believed to be a phenomenon allied to the formation designated earlier by the writer as "pectic warts." Anatomical differences occur between species; inflorescence bracts are alike throughout the genus. Wilkesia is differentiated from Argyroxiphium in its "normal" leaf structure and in its nodal anatomy. Ecological, phylogenetic, and taxonomic implications of foliar anatomy are indicated.

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