Trematolobelia: Seed Dispersal; Anatomy of Fruit and Seeds

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THE ENDEMIC HAWAIIAN GENUS *Trematolobelia* (Lobeliaceae, or Campanulaceae, subfamily Lobelioideae) was erected on the basis of its distinctive fruit. This fruit has a seed-dispersal mechanism unique in the family. Assertions have been made by some workers that holes in the fruit wall are the work of insects, and are not related to the dispersal mechanism. This contention has been adequately disproved by other investigators, but, in fact, the precise nature of the dispersal mechanism and the anatomical structure responsible for its action have never been adequately described. In addition, the present study reveals the potential taxonomic use of capsular anatomy, a feature of importance because various authors recognize one, two, or three species in the genus. These species are based largely on floral features or foliar characteristics, and not on those of the fruit. Unusually good material of *Trematolobelia* collected by the writer during the summer of 1958 provides a sufficient basis for presenting the features mentioned above in some detail.

MATERIALS AND METHODS

The following specimens were utilized for this study: *Trematolobelia macrostachys* var. *macrostachys* Zahlbruckner: Carlquist 563 (Puu Kukui, Maui); Carlquist 612 (Huumulu Rd., Hawaii); Flavious Peter April 21, 1958 (Molokai). *T. macrostachys* var. *kauaiensis* Rock: Carlquist 508 (Pihea, Kauai). *T. macrostachys* var. *grandifolia* Rock: Carlquist 612A (Cultivated at Volcano, Hawaii; plant brought from Kehena Ditch Trail, Kohala Mts., Hawaii, by Mrs. Ella Stephens).

All of these specimens were collected in the field; portions of each were preserved in

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formalin-propiono-alcohol (Johansen, 1940). Other portions were dried. Herbarium specimens of all of these collections are located in the Rancho Santa Ana Botanic Garden Herbarium.

The flowers and fruits which were studied (see figure legends for specimens used) were embedded in paraffin according to the tertiary-butyl alcohol technique of Johansen (1940: 130–131), sectioned, and stained with a safranin-fast green combination corresponding to Northen's modification of Foster's tannic acid–ferric chloride method (Johansen, 1940: 92–93).

ANATOMICAL DESCRIPTIONS

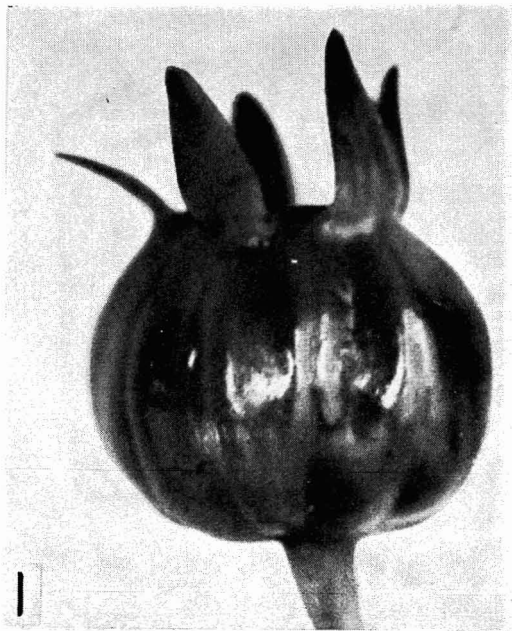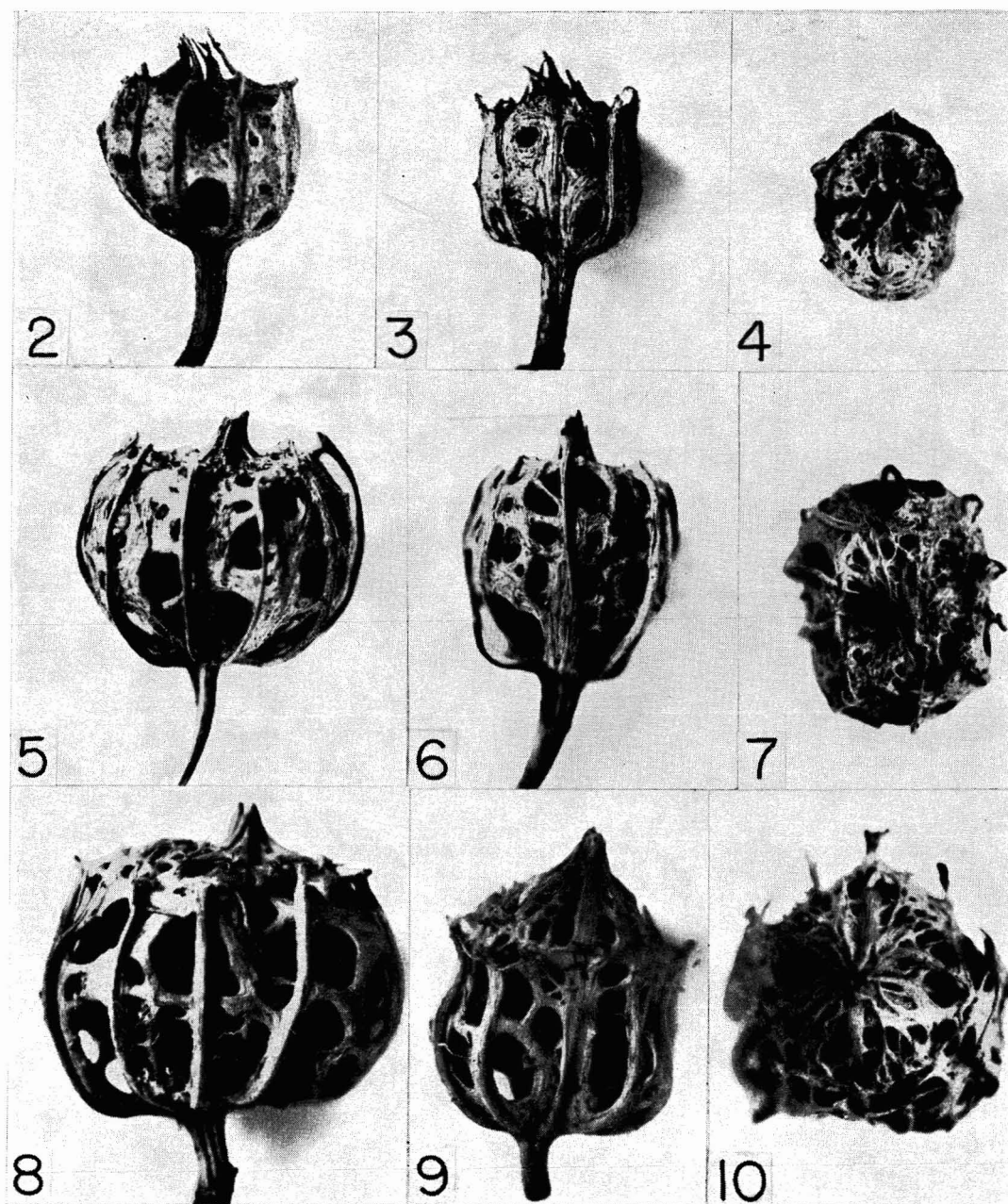The mature fruit prior to parenchyma decomposition is green and appears fleshy, much

FIG. 1. *Trematolobelia macrostachys,* Carlquist 612. Mature fruit before decomposition of parenchyma.

FIGS. 2–10. *Trematolobelia macrostachys* fruits after decomposition of parenchyma. In each row, view at left represents lateral view (with regard to carpels), middle view represents dorsiventral view (center is median line of carpel), and view at right represents top of capsule. 2–4, *T. macrostachys* var. *kauaiensis,* Carlquist 508. 5–7, *T. macrostachys* var. *macrostachys,* Carlquist 563. 8–10, *T. macrostachys* var. *grandifolia,* Carlquist 612A. For comparisons, see text. All × 3.6.

like the baccate fruits of the other endemic Hawaiian lobeliads, such as *Cyanea* and *Clermontia* (Fig. 1). Sections of this fruit (Figs. 11, 12, 15) reveal three distinctive tissues: ground-tissue parenchyma, endocarp sclerenchyma, and fibrous vascular bundles. The ground tissue of the fruit wall is composed of thin-walled parenchyma cells which are large in diameter (decreasing in size toward interior and exterior of the fruit wall). These parenchyma cells are rich in chloroplasts. Although the inner epidermis of the fruit wall is thin-walled, there are two to four layers of thick-walled sclereids internal to the epidermis (Fig. 15). As shown in Figures 11 and 12, this endocarp sclerenchyma is present around the inner surface of the carpels with the exception of the basal portion of the carpels and the portion lying between the two placentas. The apical portion of the carpels is not coated with endocarp sclerenchyma, but possesses instead a spongy sclerenchyma (Fig. 11; Fig. 17) which connects endocarp sclerenchyma with the vascular bundles which form a pointed terminus, as seen in a dry fruit (e.g., Fig. 5, above). The vascular tissue is composed of two sorts of bundles: the 10 main bundles which extend upwardly into the calyx, corolla, and stamens of the flower, and the carpellary bundles, internal to the 10 main bundles. The carpellary bundles form a dense mesh, in which large pores are present. The distinctions between the two sorts of bundles can be seen especially well in Figs. 5–7, where they are adnate only to a limited extent. The drawing in Fig. 11 shows the carpellary bundles united to the 10 vertical bundles only in the basal portion of the fruit. Thus, they appear largely separate in Fig. 12. The 10 main bundles represent the fusion of bundles from the three outermost whorls of the flower. They separate into calyx, corolla, and stamen traces only at the top of the ovary (Fig. 11, upper right). The carpellary bundles form a network-

like system which encloses the carpels. This network, seen in Figs. 2–10, is composed of bundles which run in all directions, and are absent where pores are formed. These pores are preformed in the fruit (e.g., the space between bundles at left and right, below, in Fig. 15, will be such a pore). The carpellary bundles not only form a network on the outside of the carpels, but between them (Fig. 12) as well. These central carpellary bundles supply the two placentas. In their upward extent, carpellary bundles supply the style.

The vascular bundles, both inner carpellary and outer calyx-corolla-stamen bundles, show a peculiar feature of construction. The prominence and persistence of these bundles, as seen following the decomposition of the parenchymatous portion of the fruit, is due to abundance of fibers present in these bundles. Only the basal portion of calyx-traces and style-traces possess such fibers, and entire traces are thus absent in the dry fruit. Such thick-walled fibers may be seen in Fig. 15. One might suppose that these fibers have the same origin as the fibers in most bundles, that is to say, from a bundle-cap, or protophloem region. This is, however, not the case. Sections of the ovary wall from flowers at anthesis (Fig. 16) reveal clearly that the bundles are amphicribral in construction. The periphery of the bundle consists of phloem, in which many articulated non-anastomosing laticifers are embedded. These laticifers, although present with particular prominence at the periphery of the bundles, also extend into the ground tissue of the ovary wall (Fig. 15, above left). This close association between laticifers and phloem is frequent in dicotyledons. The center of the bundle, as shown in Fig. 16, contains mature tracheary elements. Longitudinal sections of bundles reveal that these are mostly vessel elements. Between the phloem and the tracheary elements is a zone which consists

FIGS. 11–14. *Trematolobelia macrostachys* var. *macrostachys*. 11, 12, sections of fruits from the collection Carlquist 612. 11, Median longitudinal (sagittal) section of mature fruit before decomposition of parenchyma. 12, Transverse section. Conventions as follows: broken lines = tracheary elements of vascular bundles; stippled = xylary fibers; cross-hatched = spongy sclerenchyma (see Fig. 17); spatter pattern = endocarp sclerenchyma; phloem, laticifers, parenchyma, and seeds are left white. 13, 14, Fruits, following decomposition of parenchyma, dissected to show the endocarp sclerenchyma sacs; sclerenchymatous vascular tissue black, endocarp sacs stippled; from the collection Carlquist 563. 13, Lateral view, showing sacs shrunken away from base, slits below. 14, View from below, vascular tissue cut away; note slits in sacs, which permit escape of seeds. 11–12, × 5.4. 13–14, × 4.
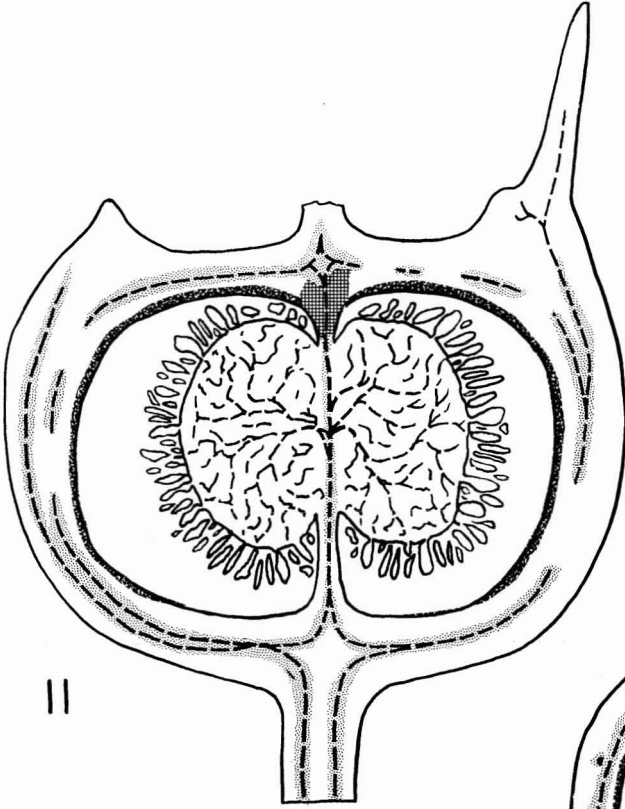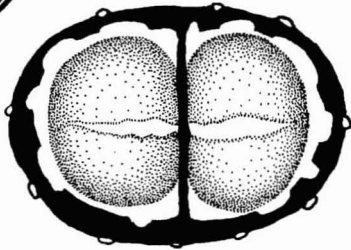
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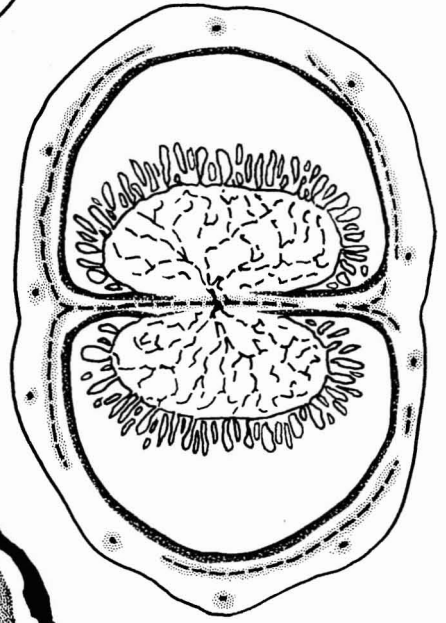
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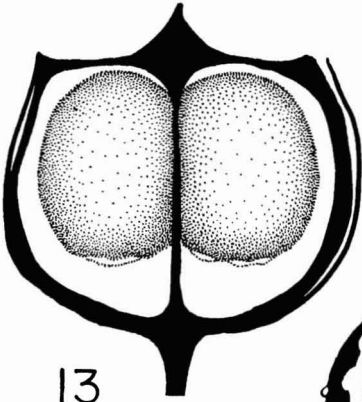
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wholly of fiber initials. Thus, the fiber initials occur internal to the phloem, and must be regarded as *xylary* fibers. As seen in Fig. 16, vessels are actually scattered among these xylary fibers. Xylary fibers are not infrequent in primary bundles, but the prominent representation of them in the vasculature of *Trematolobelia* fruits is truly exceptional. Moreover, by greatly widening the bundles, these fibers are responsible for the alteration of the carpellary vasculature from a reticulate pattern (like that in a leaf) to a sheet-like conformation, interrupted by the pores.

Trematolobelia is also distinctive among lobeliads in the prominent wings which are present on seeds. Because of the importance of winged seeds to the dispersal mechanism, these have been studied anatomically (Figs. 18–20). The central portion of the seed (Figs. 18, 19) consists of endosperm in which the embryo is embedded. At either end of the endosperm there is a haustorium. These haustoria are termed micropylar and chalazal, respectively. In details of embryo, endosperm, and haustoria, seeds of *Trematolobelia macrostachys* appear to agree with the descriptions given by Hewitt (1939) for *Lobelia amoena.* Earlier stages in embryo-sac development were not studied. The micropylar portion of the ovule is much more prominent than in *Lobelia amoena,* however. This is probably due to the formation of a wing in the seed of *Trematolobelia.* The chalazal portion likewise forms a prominent thin wing (Fig. 18, right), which is widened somewhat at the margin. This widened portion contains the ovule trace. The epidermis contains prominent thickenings worthy of description. As seen in Fig. 20, these thickenings occur on radial walls of the epidermis, and are particularly conspicuous near the outside of the epidermis. Thus, a heart-shaped outline is formed by the thickenings of adjacent cells. These thickenings do not correspond to those in the epidermis of *Lobelia*

amoena seeds. The figures of Hewitt (1939) for that species show an ellipsoid thickening in the center of radial walls of the epidermis. Rather, thickenings in *Trematolobelia* are like those figured for *Campanula sarmatica* (Campanulaceae), *Linaria vulgaris* (Scrophulariaceae), and especially *Gentiana lutea* (Gentianaceae) by Guttenberg (1926: 204–205). Portions of epidermal walls which are not prominently thickened are nevertheless thicker than walls of internal ovular cells (except for the outer epidermal wall), and bear prominent pits, both in the genera listed above (Guttenberg, 1926) and in *Trematolobelia.* Thus, anatomy of the seed-wing in *Trematolobelia* shows more similarity to that of winged seeds in unrelated genera than to that of seeds of *Lobelia,* or even non-winged species of *Campanula* (Netolitzky, 1926: 305). These thickenings undoubtedly offer mechanical strength necessary for increasing rigidity, without greatly increasing weight, in the otherwise delicate wings.

DISPERSAL MECHANISM

The controversy over the distinctness of the dispersal mechanism in *Trematolobelia,* although presumably now resolved, suggests that an accurate description of the mechanism, with comparisons with accounts of earlier authors, is desirable. Zahlbruckner's (1891) erection of *Trematocarpus,* a name later changed by him to *Trematolobelia* for reasons of nomenclatural necessity, was questioned by Hemsley (1892). Hemsley claimed that the true pores in the sclerenchymatous (carpellary) system were irregular, and caused by insects. Although Zahlbruckner reaffirmed his position in a letter, quoted by Hemsley (1893), Hemsley claimed that this could not be a true dispersal mechanism at all, and was therefore an inadequate basis for erection of a new genus. Stapf (1893) maintained that the pores in the sclerenchyma are
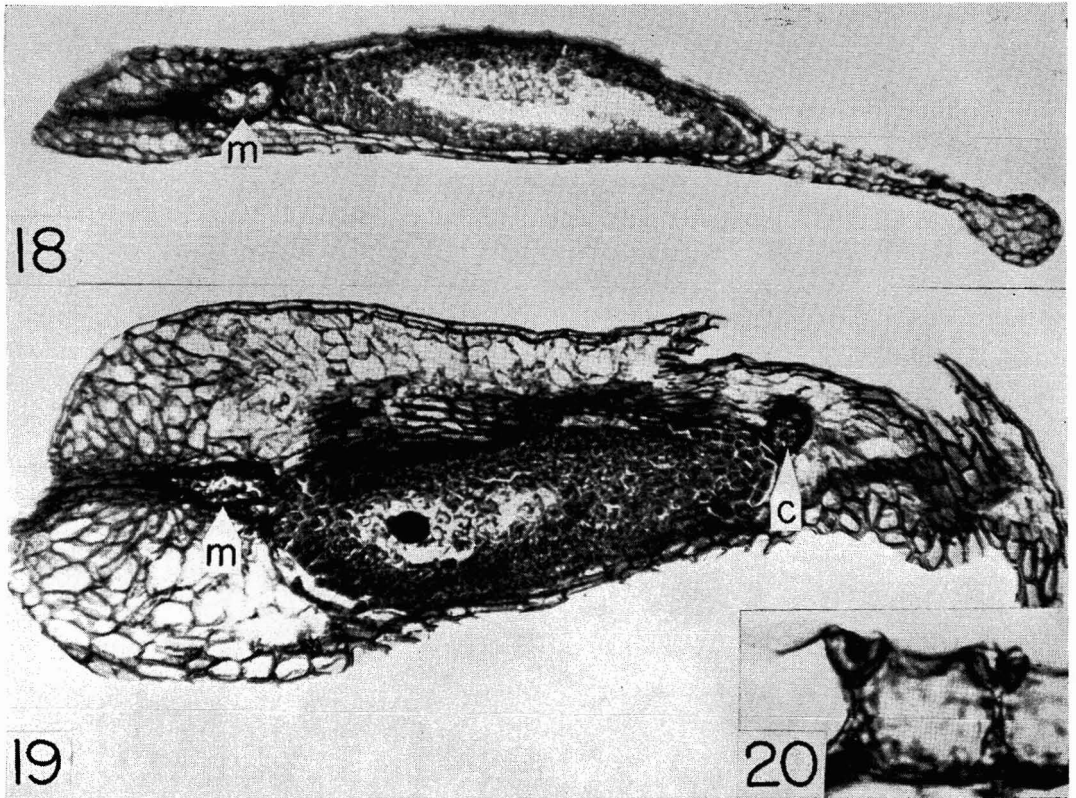

FIGS. 15–17. 15, *Trematolobelia macrostachys* var. *macrostachys,* Carlquist 612; portion of mature fruit wall, outer surface above; F = xylary fibers; L = laticifers; P = phloem; S = endocarp sclerenchyma. 16, *T. macrostachys* var. *macrostachys,* Flavious Peter IV–21–1958; portion of ovary wall, inner surface below, from flower at anthesis; the vascular bundle corresponds to the bundle shown, above right, in Fig. 16; *(F)* = future xylary fibers; L = laticifer; P = phloem; T = tracheary elements (mostly vessels). 17, *T. macrostachys* var. *macrostachys,* Carlquist 612; spongy sclerenchyma from near apex of fruit (see Fig. 11), from longitudinal section. 15, × 62. 16, × 132. 17, × 155.
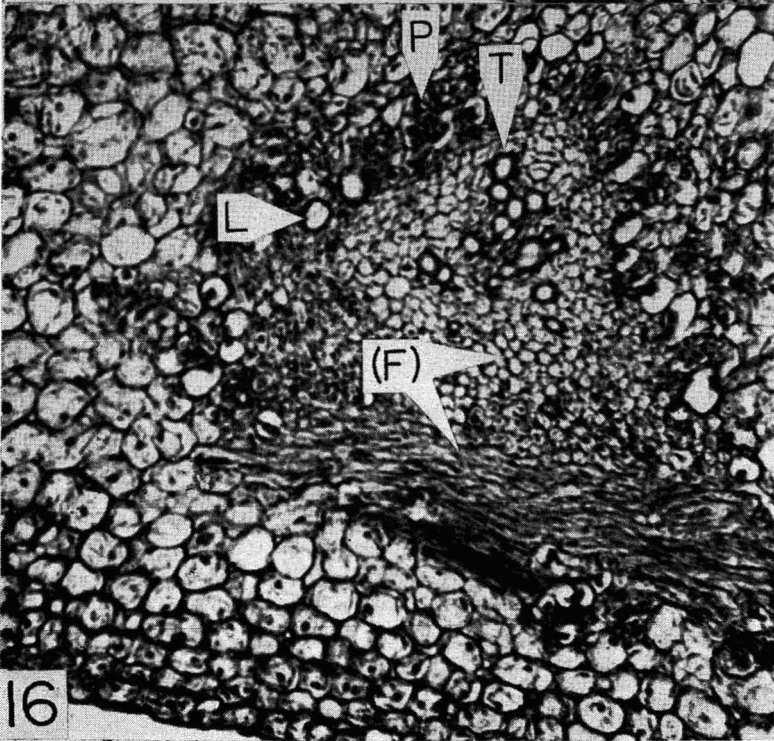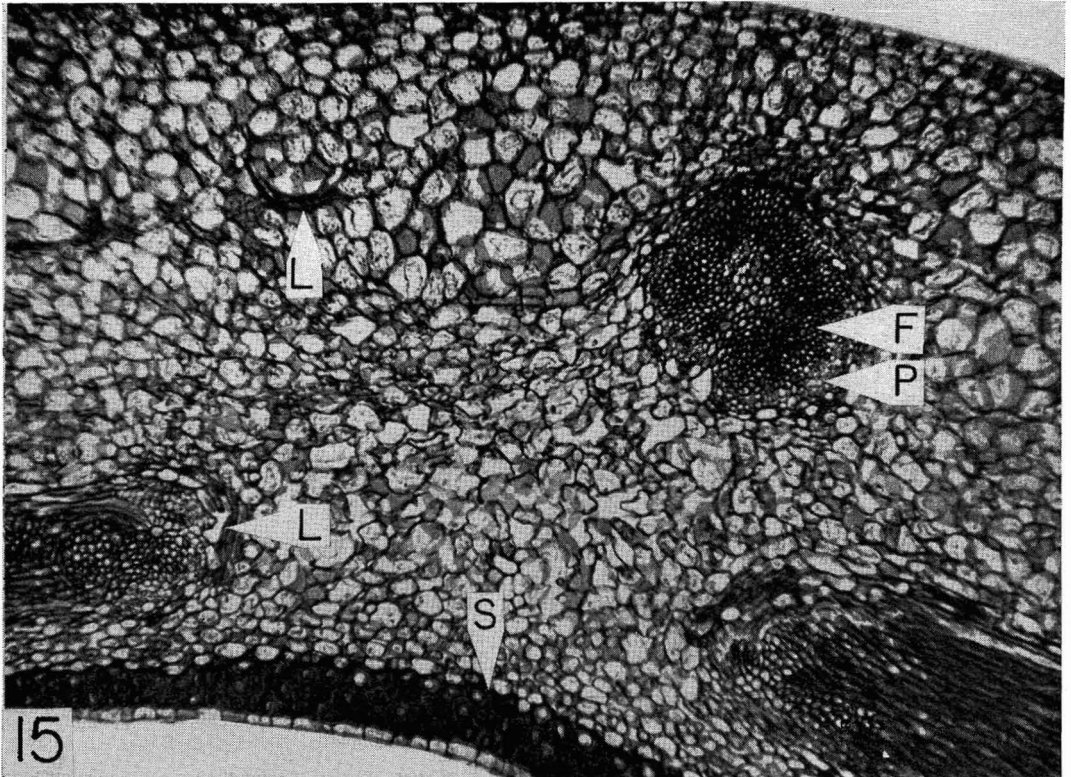
caused by insect action, but claims that similar mechanisms of dispersal in *Lobelia* (where such a sclerenchyma network is not present) negate the necessity of a separate genus for *T. macrostachys*. Rock (1919: 141) denied that insects produced holes in the capsule, and cited his own field experience in this regard. The controversy mentioned above would not have arisen if field observation had, from the beginning, been undertaken. Fortunately, I was able to study dispersal in the field and relate it to anatomy of liquid-preserved specimens.

The fully-formed capsules (Fig. 1) are borne upright on the plant. This upright position is achieved by curvature in pedicels. Such curvature takes place above the point where reduced bract-leaves are borne. This portion of the ped-

icel is thinner than that below the bract leaves, and at the time of curvature, the fibers in the pedicel vasculature have not yet formed rigid, thick walls. In July and August, the mature green capsule yellows, and the parenchymatous portion decomposes. This is not a decay caused by fungi or other microorganisms (which may, of course, occur secondarily), but rather is the result of a natural maceration. The parenchyma does not separate from the sclerenchyma by drying, as suggested by Zahlbruckner (1891) and Stapf (1893). Had these writers seen the plants in the field, they would have seen that the frequent rains in locations where *Tremato-lobelia* grows virtually prevent drying of the capsule; these rains, in fact, wash the decomposing sclerenchyma away, revealing the porous

FIGS. 18–20. *Trematolobelia macrostachys* var. *macrostachys,* Carlquist 612. Views of seeds. 18, Near-median section (embryo not shown) in a plane at right angles to wing; *m* = micropylar haustorium; chalazal edge at right. 19, Near-median section of seed, parallel to wing; *m* = micropylar haustorium; *c* = chalazal haustorium; black in center of endosperm = embryo. 20, Epidermis of seed, from chalazal end of seed sectioned as in Fig. 18; note prominent thickenings near outer surface (above). 18–19, × 90. 20, × 633.

sclerenchyma. Pores are not formed during disappearance of the parenchyma, as claimed by Zahlbruckner (1891). Rather, they are preformed, and loss of the parenchyma exocarp merely exposes these patterns. Views of the sclerenchyma network are shown in Figs. 2–10 for three collections of *Trematolobelia.*

The porous sclerenchyma varies in number and size of pores. The collection shown in Figs. 2–4 has fewer, smaller pores than those of the collection shown in Figs. 5–7; the largest pores, however, are shown by capsules of the collection illustrated in Figs. 8–10. The apex of the capsule may be composed of tooth-like structures, separate at their tips, as shown in Figs. 2–4, or as illustrated in *T. macrostachys* var. *kaalae* by Degener (1936). Other collections (Figs. 5–10), however, show that the apical portion of the capsule consists of a closed network of bundles. The pores are smaller than those in the lateral portions of the capsule.

Within the sclerenchymatous network, parenchyma around the endocarp sclerenchyma decomposes at the same time as that external to the network. The endocarp thus exposed does not have slits or pores at its apex, which is connected with the network above by the persistent spongy sclerenchyma. During occasional dry periods in the rain forest where *Trematolobelia* grows, the thin, papery endocarp can dry. Drying of the endocarp results in shrinkage, so that the sacs shrink upward from the base of the capsule (Fig. 13), and the splits in the basal and placental regions (Fig. 14) become prominent. Through these slits, the winged seeds escape. This escape is not rapid, and a few seeds may be found in capsules which are a year old. The endocarp, which does not have splits above, is apparently functional in preventing wetting of the undispersed seeds. Alternate wetting and drying can result in successive dispersals of seeds over a longer period of time, so that the entire contents of the capsule may be slowly lost. Because capsules are borne upright, and because splits occur in the basal and central portions of the endocarp, seeds are probably scattered mostly through the most basal pores in the sclerenchyma network, and the upper holes do not function appreciably in the dispersal process. I was able to demonstrate this experimentally with the capsules illustrated in Figs. 8–10, which were full of seeds when collected. Presumably the sclerenchyma network can, or does, slow seed dispersal somewhat, especially if the pores are relatively small. Undoubtedly the shaking afforded by winds, as actually observed in the field, does promote escape of seeds through the pores.

The winged nature of seeds undoubtedly is probably effective in permitting more widespread distribution (presumably by wind) of seeds. Thus, of the Hawaiian species of lobeliads studied by Rock (1919), only one, *Trematolobelia macrostachys* (the only species of the genus in Rock's treatment), occurs on all major islands. Within each island, *Trematolobelia* is present in many wetter areas of the rain forest, and although it is never abundant in a particular locality, few suitable areas seem to lack it altogether.

TAXONOMIC CONCLUSIONS

Rock (1919: 141–148) recognizes one species of *Trematolobelia* with three varieties. Degener (1934, 1936) recognizes three species, one of which is considered to have two varieties. Wimmer (1953) reduces one of these species to a variety, but otherwise follows Degener's treatment. More information obviously is needed before a clear-cut designation can be made as to the rank of entities involved. For this reason, the conservative treatment of Rock (1919) is used here. However, morphology of the dry capsules seems to be singularly neglected. Capsules are, in fact, not often collected. The taxa which have been named depend for their recognition primarily on floral characteristics, and secondarily on those of leaves. The three collections for which capsules are illustrated here represent the three major taxa: *T. macrostachys* var. *kauaiensis* (Figs. 2–4), *T. macrostachys* var. *macrostachys* (Figs. 5–7), and *T. macrostachys* var. *grandifolia* (Figs. 8–10). Distinctions among these include formation of an apical network (Figs. 7, 10) or separate teeth (Figs. 2–4), comparative size and frequency of holes in the sclerenchymatous network, total size of capsules, and relative union of the carpellary network to the 10 vertical bundles. These latter bundles are

united with the network in var. *kauaiensis* (Figs. 2–4), are largely separate in var. *macrostachys* (Figs. 5–7), and are united, but with prominent free tips in var. *grandifolia* (Figs. 8–10). None of the differences mentioned may, when more collections have been made, prove to be entirely constant, but the fact that such prominent differences occur suggests that features potentially valuable to the taxonomy of this genus deserve further investigation.

Detailed anatomical studies on capsules of other genera of Lobeliaceae may also benefit systematics by demonstrating the relationships of *Trematolobelia*. Such studies can probably aid in assessing the relative merit of various hypotheses, such as that of Stapf (1893) that *Trematolobelia* is close to *Lobelia*, or that of Wimmer (1953: 754) that *Trematolobelia* should be grouped with *Sclerotheca*.

REFERENCES

DEGENER, OTTO. 1934. *Trematolobelia.* In: The New Illustrated Flora of the Hawaiian Islands, Book 2. Privately published by the author.

——— 1936. *Trematolobelia sandwicensis* var. *kaalae* Degener. In: The New Illustrated Flora of the Hawaiian Islands, Book 3. Privately published by the author.

GUTTENBERG, HERMANN VON. 1926. Die Bewegungsgewebe. In: K. Linsbauer, ed., Handbuch der Pflanzenanatomie 5(1): 1–289. Gebrüder Borntraeger, Berlin.

HEMSLEY, WILLIAM B. 1892. *Trematocarpus.* Ann. Bot. 6: 154.

——— 1893. The genus *Trematocarpus.* Ann. Bot. 7: 289–290.

HEWITT, W. C. 1939. Seed development of *Lobelia amoena.* Jour. Elisha Mitchell Sci. Soc. 55: 63–82.

JOHANSEN, DONALD A. 1940. Plant Microtechnique. McGraw Hill, New York. xi + 523 pp.

NETOLITZKY, FRITZ. 1926. Anatomie der Angiospermen-Samen. In: K. Linsbauer, ed., Handbuch der Pflanzenanatomie 10(1): 1–364.

ROCK, JOSEPH F. 1919. A monographic study of the Hawaiian species of the tribe Lobelioideae, family Campanulaceae. Mem. Bernice P. Bishop Mus. 7(2): 1–394.

STAPF, OTTO. 1893. The genus *Trematocarpus.* Ann. Bot. 7: 396–398.

WIMMER, F. E. 1953. Campanulaceae-Lobelioideae, II Teil. In: R. Mansfeld, ed., Das Pflanzenreich 107(2): 261–813. Akademie-Verlag, Berlin.

ZAHLBRUCKNER, ALEXANDER. 1891. Ueber einige Lobeliaceen des Wiener Herbariums. Ann. Naturh. Hofmus. Wien 6: 430–445.