

A Preliminary Morphological Study of *Epifagus virginiana* (L.) Bart.

A. E. BROOKS, Wabash College¹

A parasitic plant common to the Eastern United States is *Epifagus virginiana* (L.) Bart. Linnaeus first described it under the name of *Orobanche virginiana* in 1753. Dr. Herman Schrenck (6) made the first detailed study of *Epifagus* in 1894. His work was physiological in nature, being concerned with the parasitism of the plant, although some emphasis was given to the anatomy and general morphology. In 1895, Dr. Macfarlane, of the University of Pennsylvania, began a study of *Epifagus* which was completed by Ethel Cooke and Adeline F. Schively (2) in 1904. Their work dealt with the general structure of the plant, emphasizing the organization and development of the flower. The most recent study of the Orobanchaceae of which *Epifagus* is a genus was conducted in 1920 by Irwin Boeshore (1). He presented an account of the general morphology and histology of the root, along with a comparative study of the stem, leaf, and flower parts including the nectaries and seeds of other species of the Orobanchaceae and the semiparasitic Scrophulariaceae. With the information presented in the aforementioned papers in mind, it is the aim of this author to contribute to the general knowledge of the morphology, especially the anatomy, of this common woodland plant.

Most of the plants used in this study were collected in the W. C. Allee Memorial Woods, Parke County, Indiana. A few very young developmental stages were collected at Ringwood near Ithaca, New York. *Epifagus* can usually be found growing in the thick duff at the base of, or in the near proximity of, *Fagus grandifolia*. Some specimens, however, have been found quite distant from beech trees and on slopes where a duff was absent. All specimens were preserved in FAA immediately after collection, and then sectioned and stained, using Safranin O and Fast Green, according to standard methods of plant microtechnique (3).

At maturity, the branched plant ranges from twelve to eighteen inches in height, and usually appears inconspicuously light brown in color. In some areas the abaxial surface of the lateral branches is a dull purple in color indicating a concentration of anthocyanin. Pease (5) reported a form which was, except for the corolla, purple throughout, while Palmer (4) reported a population in which the individuals were butter yellow in color. These forms are evidently the exceptions and are not consistent with those plants collected by the author.

The leaves of *Epifagus* are reduced to small tooth shaped scales arranged in a 2/5 phyllotaxy about the aerial portion of the main axis. Stomata are present on the scales, and multicellular hairs are borne along the edges of the scales, but neither are numerous.

By digging within the duff in late August, beneath plants of the previous year, it is possible to find young plants in various stages of

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development. These younger forms have an enlarged basal portion which at maturity is the subterranean tuber. On the enlarged portion of many, fragments of beech root are often evident. At this young stage, the plants are commonly white to butter yellow in color, and may range in size from one millimeter to several centimeters.

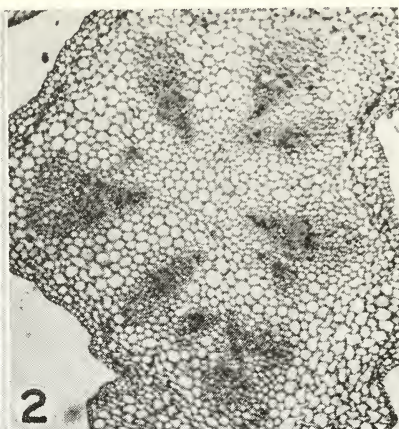
A study of sections through the tip of the stem shows that groups of apical and subapical cells constitute the initiating body of the stem. The initials appear to be in three tiers, the cells of the outer one dividing anticlinically and those of the inner ones dividing in all planes.

Cross sections of the stem reveal an internal anatomy common to most herbaceous dicotyledons. A relatively thick waxy cuticle appears to envelop the epidermis of the stem. The regular epidermis is composed of narrow cubical cells; stomata and multicellular trichomes appear at infrequent intervals along the stem. Large, elongate parenchyma cells with numerous intercellular spaces make up the relatively narrow cortical region of the stem. These cells are usually devoid of starch grains at maturity.

Contrary to what was reported by Cooke and Schively (2) and by Boeshore (1), this worker found the vascular system to consist of a definite ring of bundles (Fig. 2), not scattered bundles as was previously reported. It is a dictyostele made up of bicollateral bundles since a small amount of internal phloem has been detected in most sections. The number of bundles in the mature stem usually ranges somewhere between nine and thirty. Each bundle is capped by a mass of sclerenchyma cells, fibers, toward the outside of the axis. The phloem accounts for about one third the size of each bundle. Since a defined pericycle and endodermis as well, are absent, the outer portions of the bundle caps could be considered a pericyclic region. There appears to be no evidence to indicate the presence of a vascular cambium and hence all growth is primary.

The phloem consists of sieve tube elements and companion cells, and exceeds the xylem in mass to a greater or lesser extent. Boeshore (1) considered this reduction in the amount of xylem a further advance in degradation which accompanies an increased parasitic habit. Compound sieve plates have been observed on the oblique end walls of the sieve tube elements. The cells of the phloem are relatively small, and angular in cross section, although a few large spherical cells have been spotted in this region. These cells appear to be phloem parenchyma. This author is in disagreement with Cooke and Schively (2), as well as Boeshore (1), who report, "an internal phloem is almost always present, often in excess of the outer phloem mass." In many sections, including those of various developmental stages, through a great number of locations along the axis,

Fig. 1-6 *Epifagus virginiana* (L.) Bart. Fig. 1.—Longitudinal section through ovule showing microphyllar haustorial endosperm. x43. Fig. 2.—Cross section of mature stem showing ring of bicollateral bundles. x5. Fig. 3.—Longitudinal section of older tuber showing region of attachment with *Fagus grandifolia*. x5. Fig. 4.—Longitudinal section of young tuber showing haustorium penetrating beech root and converging xylem masses. x10. Fig. 5.—Longitudinal section of young plant showing a well differentiated haustorium and beech root in cross section. x10. Fig. 6.—Longitudinal section showing same features as fig. 5. x10.



the internal phloem never exceeds the outer phloem in mass and in fact is always present in very small amounts. The internal phloem is at times, depending on the age and location along the axis, almost indistinguishable from the cells of the outer pith. The structure and cell type of the inner phloem mass is similar to that of the external phloem.

The xylem is composed of tracheids, mainly scalariform pitted, and vessels. The length of the vessels greatly exceeds that of the tracheids, and they show a porous end wall condition as well as annular, loosely spiraled, tightly spiraled, and scalariform elements, indicating protoxylem and rapid growth. The protoxylem elements appear consistently nearer the center of the axis, and this establishes an endarch arrangement and centrifugal development. The xylem of the mature stem is easily recognized by the thick walls of its cells and usually consists of two or three cell rows. These cells are much larger than those of the phloem, and nearly as large as those composing the bundle caps in size and shape, when seen in cross section.

Occupying the center of the axis are the large elongate parenchyma cells of the pith. Similar to the cortical region, many intercellular spaces are present in the pith. The cells of this group are commonly larger than the cortical parenchyma, often reaching their greatest size in the pith rays which extend outward to the cortex, between the vascular bundles. Unlike the cortical cells, the cells of the pith usually contain numerous starch grains. The quantity of starch decreases in the pith ray cells from the pith to the cortex.

The tuber, from which the erect stem arises, consists of an elongated subterranean structure ranging between one and four centimeters in length. It is covered with numerous small stiff branched structures. Schrenk (6) referred to these structures as grapplers. This underground structure seems to be similar to the Irish potato tuber. Boeshore (1) looked upon the tuber as a fused primary root below and a greatly condensed vegetative stem-axis above. A section through the tuber reveals an anatomy somewhat different from the arrangement found in the stem. A single layered epidermis very similar to that of the stem forms the outer covering of the tuber. Just beneath the epidermis is a narrow band of parenchyma cells corresponding to the cortical cells of the stem. In the center of the tuber are larger parenchyma cells like those of the stem pith. Unlike the condition found in the erect stem, starch grains are uniformly present in all but the vascular cells of the tuber.

Although there is a similarity of cell type between the phloem of the stem and that of the tuber, the phloem in the tuber follows a very irregular branching pattern. In both cross and longitudinal sections it appears not to be closely associated with the xylem. The xylem masses in the tuber are arranged in a ring, like the bundles in the stem. Each of these masses appears to converge toward the point of attachment with the beech root. Just within the *Epifagus* tuber near the point of attachment with the host, the xylem appears as a single large mass. The xylem masses present in the tuber, but distant from the point of attachment, are rarely more than four or five cells in width and are usually cubical reticulate tracheids. Bundle caps, when present, are always smaller in mass than those present in the stem.

The actual root or grappler is a short stiff branched structure at maturity and is supplied with a single mass of vascular tissue, which seems to represent a stele. The xylem of this mass rarely consists of more than a single row of tracheid, which suggests a diarch arrangement. Each root arises in the pericyclic region of the tuber and forces its way outward through the cortex and epidermis. Although these structures lack root caps, they possess other characters which strongly suggest that they are true roots. This worker has no basis to disagree with Boeshore (1) who reports that these structures are functionless due to the high degree of parasitism exhibited by *Epifagus*.

Although many specimens that were collected appeared to be growing independent of the beech, all showed a definite attachment to the beech root when examined microscopically, regardless of age. In older stages of *Epifagus*, the beech root appears deeply or shallowly buried within the tuber, as has been reported by previous workers (1) (2). The author agrees with Boeshore who suggested that this condition arises by the development of the tuber tissues around the beech root, eventually both becoming confluent; but there is disagreement as to when the actual connection has been established. Boeshore contended that the connection is established when the *Epifagus* tuber has partially or totally enveloped the beech root. This suggests that there is a time during the young developmental stages of *Epifagus* when it lives independently. More conclusive evidence has been found by sectioning very young forms of *Epifagus*.

These sections reveal that a definite haustorium is formed within the parasite (Figs. 4, 5 & 6). It develops outward and pierces the small beech root thus establishing the union between host and parasite at a very early stage in the development of the latter. This haustorium-like organ has not been reported by any of the past workers. As the tuber develops around the beech root the haustorium becomes less and less evident. (Figs. 4, 5 & 6). At maturity the haustorium of *Epifagus*, in most cases, is not evident (Fig. 3).

The inflorescence of *Epifagus*, formed at maturity, is a spike, in which the upper flowers are sterile. Each of the lower fertile flowers contains four stamens and a pistil. The style is relatively short and merges with a large globose ovary. The ovary and style are glabrous and the stigma shows a much branched condition. The numerous ovules within the ovary, which is composed of four fused carpels, show a shallow axile placentation. Enclosing the pistil and stamens are the modified corolla and calyx which resemble the rest of the plant in color. The flower is undoubtedly a cleistogamous one with self-pollination occurring.

Shortly after fertilization a micropylar haustorial endosperm is evident (Fig. 1) and it later develops into the cellular endosperm of the seed. The fertilized egg undergoes divisions forming a several celled undifferentiated embryo at the time of seed shedding in the late fall. The integuments undergo modification forming a tough, heavily lignified, ridged seed coat. The seeds are elongate and quite small, less than one half a millimeter in length. Cooke and Schively reported that between 700 and 1800 seeds are formed by the plants. (2)

After examination of soil samples collected beneath dead plants of the previous growing season, many seeds shed the past year were obtained.

Sections of these seeds revealed an internal condition very different from that present at the time of shedding. The embryo had become differentiated into a many celled body which was enlarged at one end and tapered abruptly into a nearly filamentous structure at the opposite end. The cells of the endosperm had also become modified, containing irregular masses of granules. These aggregations of granules seem to represent some sort of stored food. The condition of the embryo of *Epifagus* indicates that it undergoes a development similar to the embryos of many kinds of orchids. Differentiation does not occur until the seeds have been shed and have passed through a period of dormancy.

There are many phases of the life cycle of *Epifagus* which have not been thoroughly investigated, especially those stages between fertilization and germination. In spite of the fact that there are several points of difference between the results presented here and previous investigations, it can be concluded that the general morphology indicates, without question, that *Epifagus virginiana* is truly a parasitic plant.

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