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The History of Germinating Embryos of the Eastern Dwarfmistletoe (Arceuthobium pusillum Peck)

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The Histology of Germinating Embryos of the Eastern Dwarfmistletoe (Arceuthobium pusillum Peck)[1]

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ABSTRACT — Embryos of germinating seeds of *Arceuthobium pusillum* have four histological zones: the protoderm of the radicular apex, which remains distinct throughout germination and through anticlinal divisions gives rise to the epidermis; the procambium, which matures into a simple vascular strand in the mature radicle; the ground meristem, which matures into the cortex; and the promeristem, from which the procambium and ground meristem are derived.

The commercial black spruce forest type occupies approximately 1,400,000 acres in northern Minnesota (Stone, 1966) and, as such, represents a rather sizable reservoir of prime pulp timber. The spruce, on from 3-11% (Anderson, 1949), and perhaps more, of this acreage have been infected with the eastern dwarfmistletoe (*Arceuthobium pusillum* Peck) which causes the slow but constant deterioration and death of virtually all trees in constantly expanding infection centers.

Attempts at eradication of the parasite have been unsuccessful, largely because we lack basic information on its life history and ecology. To provide some of this much-needed information, the mature seed was studied in respect to environmental factors that influence germination, the histology of the mature seed, and the histology of germinating embryos.

Materials and Methods

Mature dwarfmistletoe seeds were collected from infected black spruce (*Picea mariana* (Mill. B.S.P.) trees in infection centers located in the Fond du Lac State Forest 10 miles east of Cromwell, Minnesota, on September 16, 1966. Because of the explosive nature of mature fruits and their small size, large quantities of seeds were collected from infected branches bearing mature female shoots that had not yet dispersed their seeds by covering them with a layer or two of muslin and then squeezing the covered branchlet to induce seed dispersal. It was obvious when seeds were being dispersed because of the "snapping" sound made by the exploding fruits and the detectable sound of seeds striking the muslin. The sticky viscin layer covering each seed caused it to adhere to the muslin. The seeds were removed immediately and stored at 40°F. until December, when the germination test was started. A portion of the seeds were immediately killed and fixed in formalin-acetic acid-alcohol (Johansen, 1940:41).

Water agar, consisting of 3 gm. of agar in 150 ml. of distilled water, was poured into five sterile petri dishes and allowed to solidify. One hundred cork disks 2mm. thick and 5mm. in diameter were sterilized and 20 were placed on the agar surface in each of the five petri dishes. The dishes were then stored at 25°C. for 24 hours.

On the following day, December 21, 1966, one hundred dwarfmistletoe seeds were surface disinfected by vigorously swirling them for 90 seconds in a freshly prepared 1 per cent solution of sodium hypochlorite and immediately rinsed in two changes of sterile distilled water for 30 seconds in each. One seed was placed on each disk in the petri dishes and the dishes were incubated in the dark at 25°C. with 60 per cent relative humidity.

Germinating seeds were removed at various intervals during the incubation period, killed and fixed in formalin-acetic acid-alcohol, and dehydrated according to Sass's (1951:27) slightly modified schedule. The seeds were embedded in 56°C. Tissuemat and sectioned in 10 microns thick serial ribbons on a Spencer rotary microtome. The ribbons were affixed to glass slides with Haupt's adhesive and 4 per cent formalin and left to dry for 24 hours. They were then stained wtih safranin and fast green (Johansen, 1940:80) and mounted in Permount. After drying for several weeks they were cleaned and labeled.

A Zeiss drawing tube was used for drawing representative sections of germinating embryos in various stages of development.

Results and Discussion

The development of fruits of *A. pusillum* from the time of pollination to near-maturity has been described by Thoday and Johnson (1930) and much of their terminology for the various tissues of the seed have been adopted for this analysis.

A longitudinal section of a mature seed of *A. pusillum* collected September 16 is diagramed in Figure 1. The embryo lies along the central axis of an ovoid mass of endosperm. Both were light green in color in fresh specimens indicating the presence of chlorophyll. The embryo is nearly cylindrical in shape, domed on both ends, and about 0.5 mm. long. It is slightly exserted from the enfolding endosperm. The crushed remains of the nipple (Thoday and Johnson, 1930:818) are visible at the opposite end of the endosperm. The entire mass of endosperm, embryo, and nipple is covered by the endocarp,

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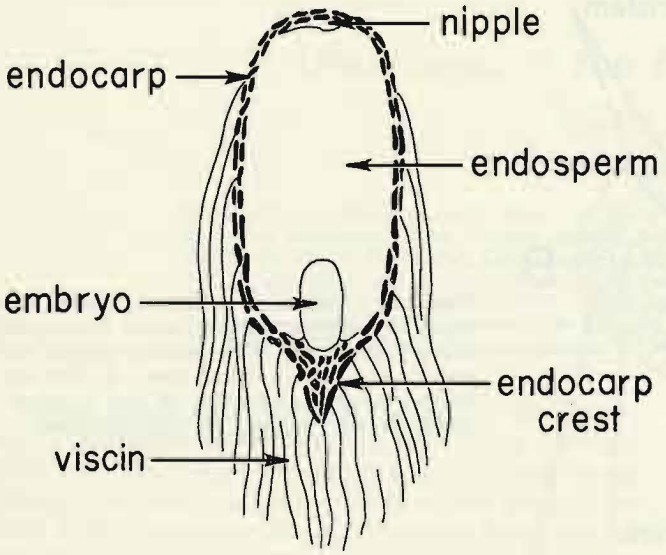

FIGURE 1. Diagram of mature seed of *A. pusillum* showing the major histological zones (26X).

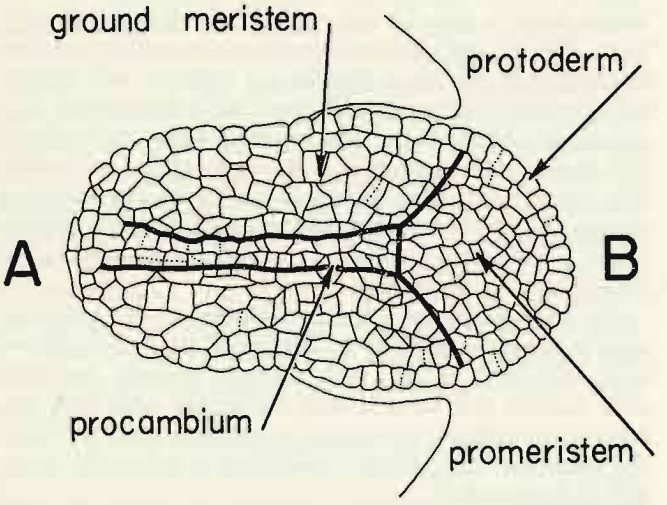

FIGURE 2. Longitudinal section of mature embryo of *A. pusillum* collected Sept. 16, showing major histological zones (150X). The most recently formed cell walls are shown as dotted lines. A.–epicotylary apex; B.–radicular apex.

which consists of large, thin-walled cells that were crushed by the expanded endosperm into a tough, almost sclerotic layer 2 or 3 cells thick. Although never directly joined to them, the endocarp apparently protects the endosperm and embryo from mechanical injury and desiccation. All of the exterior of the endocarp, except for the domed end that covers the nipple, is covered with a sheath of mucilaginous cells forming a substance called viscin, which glues the seed to its host after it has been dispersed.

The author did not observe cotyledons in embryos of *A. pusillum*, nor did Thoday and Johnson (1930), although Kuijt (1960) reported the presence of vestigial cotyledons in embryos of *A. campylopodum* Engelm., a western dwarfmistletoe. Although *Arceuthobium* itself is a very specialized genus (Kuijt, 1964), the lack of

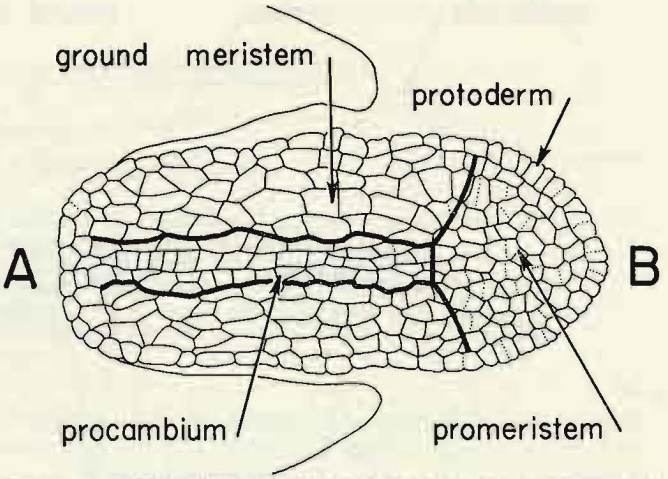

FIGURE 3. Longitudinal section of an embryo of *A. pusillum* in which germination has begun, showing major histological zones (150X). The most recently formed cell walls are shown as dotted lines. A.–epicotylary apex; B.–radicular apex.

cotyledons in *A. pusillum* may be one indication that it is one of the most highly evolved species in this genus and lost the apparently useless organs as it became more completely parasitic.

The tissues in mature embryos (Figure 2) are well defined. The entire outer surface of the embryo, except for a small portion at the epicotylary apex (A), is covered with a one cell-thick layer of protoderm. A promeristem, consisting of a loosely arranged mass of cells, has begun to differentiate just beneath the protoderm of the radicular apex (B). Very little mitotic activity is evident at this time but the promeristem region is markedly visible from the deeply stained dense protoplasmic contents of its cells, as is the procambium which has formed a core several cells in diameter through the longitudinal axis of the embryo connecting the promeristem with the epicotilary apex of the embryo. The remaining tissue surrounding the procambium is the ground meristem consisting of weakly vacuolated cells.

The percentages of seeds that germinated are given in Table 1. None had germinated after 5 days. After 10 days, 16 seeds had germinated, as evidenced by the dark red to maroon domed radicular apex emerging through the ruptured endocarp in a central or slightly off-central position. The crest of the endocarp was usually pushed aside, but occasionally was carried along with the emerging radicle. After 15 days, 60 out of the 100 seeds had

TABLE 1. Percentage germinations of seeds of *A. pusillum* incubated at 25° C. and 60% relative humidity, starting December 21, 1966.

Days	% Germination*
5	0
10	16
15	60
20	69
25	71

*Based on a total sample of 100 seeds.

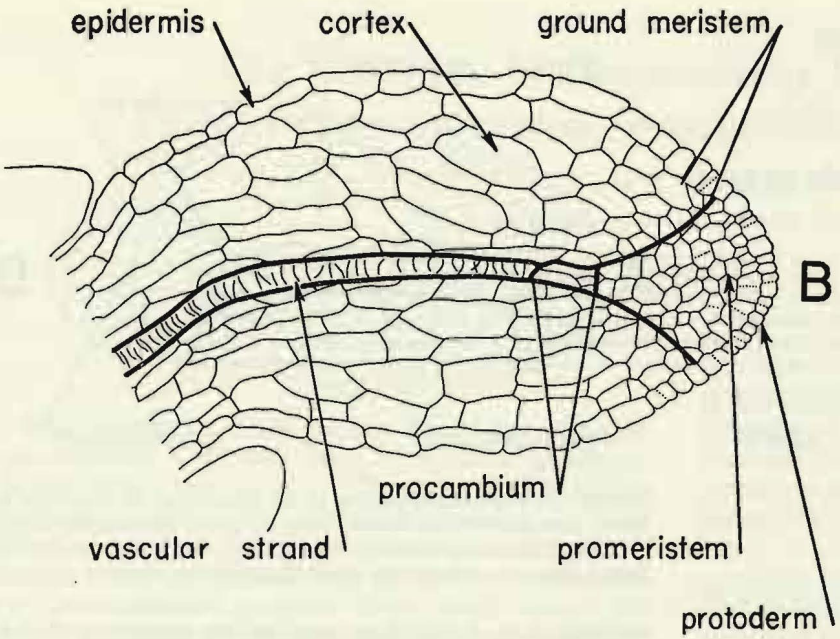

FIGURE 4. Longitudinal section of mature radicle of *A. pusillum* showing major histological zones (150X). The most recently formed cell walls are shown as dotted lines. A.–The epicotylary apex has been cut off and is not included in this diagram; B.–radicular apex.

germinated, and only 11 seeds germinated in the succeeding 10 days. The rapid decline in germination was probably a reflection of the seeds' germinative energy as fungi were not evident during any part of the incubation period.

A longitudinal section of an embryo in which germination has begun is shown in Figure 3. The lateral wall of the embryo, as a result of the elongation of cells of the protoderm, has become completely detached from the endosperm with only the epicotylary apex (A) remaining attached. After germination has started, the epicotylary apex never seems to resume meristematic activity and is probably only absorptive in function, serving to transport food materials from the endosperm to the yet unformed vascular strand, which, in turn, will carry them to the promeristem of the elongating radicle. Cells of the ground meristem have ceased mitotic activity and have become much more highly vacuolated, containing large numbers of starch grains. The promeristem is quite active and new walls are forming randomly in all directions. The protoderm has kept pace with the expanding radicle through an increase of anticlinal divisions.

By the time the radicle has grown 1mm. in length from the endosperm, all its tissues are mature (Figure 4) and are similar to those reported by Thoday (1951) for *Viscum album L.*, a species in a related genus. Much of the increase in size is the result of elongation of cells in the cortex and epidermis. This promeristem is smaller than that illustrated in Figure 3 for an earlier stage of growth but is still quite active. Immediately behind this region, the procambium has differentiated into a simple vascular strand, which is readily identifiable from the spiral thickening of its elements. The vascular strand, although not entirely shown in Figure 4, which shows only the radicle, continues throughout the longitudinal axis of the radicle and embryo and usually matures to within 4 or 5 cells of the epicotylary apex of the embryo. The xylary elements become somewhat stretched as elongation continues but do not rupture, and, apparently, remain functional until the host is penetrated. The ground meristem region is somewhat vague in its limits and grades rather abruptly from promeristem to cortex. The cortical cells have increased greatly in size and have become highly vacuolated with numerous starch grains. There is no trace of a root cap during any stage in the development of the radicle.

On fresh specimens, the protoderm on the radicular apex is marked externally by very small irregularly placed cells, each with a tiny, dark maroon vacuole. As these cells mature and enlarge into epidermal cells they become rectangularly prismatic in shape and have large, red vacuoles, the whole resembling a miniscule brick wall in appearance.

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