DEVELOPMENT OF UNPOLLINATED OVULES OF QUAKING ASPEN

Gilbert H. Fechner [1]

INTRODUCTION

The development of quaking aspen (Populus tremuloides Michx.) female flowers has been studied following wind pollination in the field (Nagaraj, 1952) and following forcing and controlled pollination in the laboratory (Winton, 1968; Fechner, 1972). However, the development of flowers of this species has not been studied in situ following controlled pollination.

Furthermore, previous work (Fechner, 1972) has shown that unfertilized ovules, within ovaries containing fertilized ovules, apparently develop to the mature embryo sac stage and degenerate soon thereafter. Whether or not development can proceed similarly in unpollinated pistils is not clear. Therefore, the objective of this study was to compare the development of ovules in unpollinated quaking aspen flowers to that in control-pollinated flowers and to relate this development to external appearances of aments and pistils.

METHODS

On March 31 and April 1, 1973, branches bearing inflorescences, were isolated on a single female quaking aspen tree with plastic bags. Approximately one-half of the flower buds were tightly closed at this time, and one-half showed bracts extending up to 1/3 bud length beyond the tips of the bud scales.

On April 12, 1973, when the majority of the stigmas on the bagged flowers were receptive, pollinations were tarred out (a few were done on April 10), randomly, on approximately one-half of the bagged branches; the other half were left unpollinated. Fresh pollen was applied with a camel's hair brush, and the bags were immediately replaced. At approximately 3-day intervals, beginning before pollination and continuing until May 11, one or more bagged branches, each of pollinated and unpollinated material, were collected.

On each collection date, approximately five aments each of pollinated and unpollinated material were killed and fixed in FAA. The lengths of five aments were measured, as were the lengths of 10 pistils of each ament. A portion of the material was dehydrated in an ethyl-butyl alcohol series and individual pistillate flowers were embedded in hard paraffin, and sectioned on a rotary microtome, at about 10 microns. Most satisfactory straining was obtained with a safranin-fast green series. Measurements of ovule and embryo length were made with the aid of an eyepiece micrometer.

[1] Professor of Forest Genetics, Colorado State University, Fort Collins, Colorado. The author wishes to thank Kristine Anderson for assistance in preparation of most microscope slides used in this study.

RESULTS AND DISCUSSION

Pre-pollination.--(March 31 to April 12) - At that time the branches were isolated, the buds were tightly closed or the tips of the bracts were exserted. Dissected buds revealed pistils (gynoecia) about one millimeter long, with the cup-like disc extending about 2/3 the distance from the base of the ovary to the stigmas. Most of the ovules at this time were less than 0.1 mm long, the integuments were just initiating, and the gametophyte was in the megaspore mother cell stage. These ovules agreed with the megaspore mother cell stage described by Nagaraj (1952) for quaking aspen and by Graf (1921) for European aspen. In some ovules the megaspore mother cell was not yet differentiated. Thus, as quaking aspen aments emerge from the buds in spring, they are not much advanced beyong their development of the previous September, as reported by Lester (1963).

During the next 10 to 12 days, i.e., up to the time that pollen was applied, the aments emerged completely from the buds, approximately quadrupling in length. The pistils doubled in length, the cup-like disc now extending only about 1/2 the distance from the base of the ovary to the stigmas. No hair cells had yet differentiated in the ovaries. Ovules increased to 0.2 mm in length, and the embryo sac (female gametophyte) reached the 4-nucleate stage at pollination; few ovules remained in the 2-nucleate stage.

During the early part of the study, the aments and pistils within the isolation bags advanced in development as much as six days beyong unbagged material. For example, peak receptivity was reached in flowers of bagged aments on April 12, compared to April 18 for unbagged flowers. Thus, elapsed time between different developmental stages as reported here may not coincide precisely with that under natural conditions.

Pollination to fertilization.--(April 14 to April 20) - During the first week after pollination, to April 20, development of pollinated and unpollinated aments and pistils was similar. The aments elongated to approximately 25 millimeters and the pistils to about 2.5 millimeters. The cup-like disc still surrounded approximately the lower one-half of the pistil; later, this structure was less prominant.

Internally, the ovules were slightly more than 0.2 millimeters long, and the embryo sac reached maturity by April 14; the fusion nucleus was formed and centrally located. Few embryo sacs were still in the 8-nucleate stage on April 20.

Just prior to maturation of the embryo sac, development of the epidermal hair cells was initiated acropetally, those originating farthest down the funiculus or in inter-funicular areas having the greatest length. This agrees with the observation of Nagaraj (1952), who stated that the epidermal hair cells are initiated at approximately the early 8-nucleate stage of the mega-gametophyte. In mature embryo sacs containing a fusion nucleus, hairs reached upward to about 1/2 to 3/4 the length of the ovules on whose funiculi they arose, as also observed by Winton (1968). More distally-originating hairs reached to near the tops of the ovules.

Post-fertilization.--(April 22 to May 11) - In a few ovules from the April 22 collection very early embryonic development was found, whereas none of the ovules from collections two days earlier had any embryos. Thus, fertilization in control-pollinated quaking aspen apparently occurred 10 to 12 days following pollination, somewhat longer than the three to five days estimated for forced branches in the laboratory (Fechner, 1972) and a great deal longer than the 24 to 48 hours commonly observed in angiosperms (Krugman, et al., 1974).

Embryos soon became globose in shape, lying at the micropylar end of the ovule, in free-nuclear endosperm. This globose embryo was of about the size previously estimated to consist of 180 to 200 cells, or eight to nine cell divisions past fertilization and produced in the laboratory 168 hours (7 days) following pollination (Fechner, 1972). There seems to be a great deal of regularity in the positioning of two free nuclei, in close association with the globose embryo, just above and slightly to each side of the young embryo. This polarity in the cytoplasm has not been previously reported, and its significance, if any, is unknown.

Pollinated aments elongated approximately 30 percent in the two days from April 20 to 22 and another 25 percent by May 1 (Figure 1), whereas unpollinated aments increased a total of about 25 percent during that time. This increase in length was due primarily to elongation of the rachis immediately after fertilization. Both pollinated and unpollinated aments elongated at a surprisingly uniform rate throughout the observation period, and only this slight post-fertilization difference produced longer pollinated aments than unpollinated ones, It is possible that elongation of the rachis is not as direct a function of pollination or fertilization as other growth phenomena are.

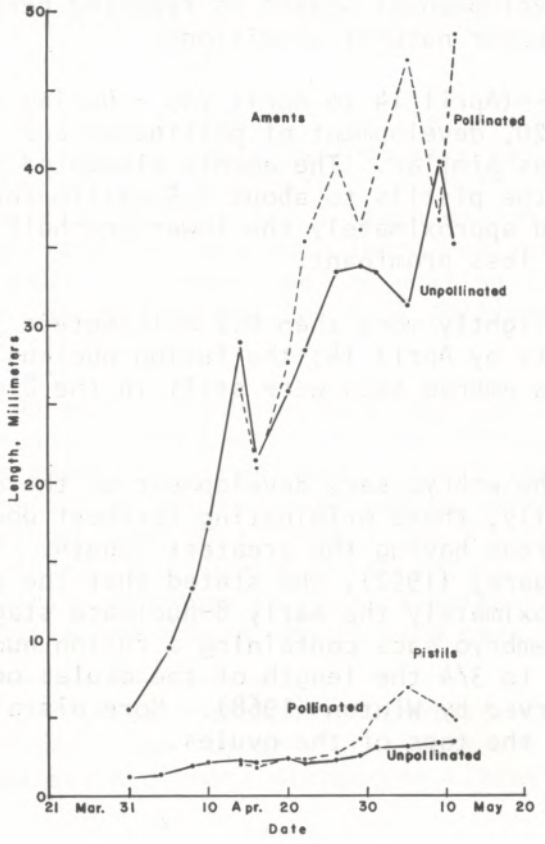

Fig. 1.--Ament and pistil length of quaking aspen, 1973. Each point based on measurement of 5 aments, or on 10 pistils per ament.

The pistils of pollinated and unpollinated flowers were not noticeably different in length or other appearance by April 22, the approximate fertilization date, But by May 1 the pollinated pistils had doubled in length to over 5 mm, about twice as long as the unpollinated pistils; the stigmas of both had dried or were drying.

Hair cells continued to develop in both pollinated and unpollinated material so that in two days after the first embryos were observed, they reached to the tops of the ovules and in 10 days they filled the pistils into the styles. These funicular hair cells eventually form the tuft or coma attached to remnant funiculi on mature seeds (Strain, 1964; Krugman, et al., 1974),

The ovules in both pollinated and unpollinated material, including those with initial embryonic development, were also still about equal in length up to April 22. They had not elongated since reaching the 4-nucleate embryo sac stage about 10 days earlier, but by May 1, the pollinated ovules had more than tripled in length. Little additional embryo growth occurred in this material, however, and the three-fold increase in ovule size was primarily vested in enlargement of the free-nuclear endosperm. In early stages, endosperm formation commonly proceeds more rapidly than embryo development in angiosperms (Krugman, et al., 1974).

Unpollinated ovules, did not increase in length from that reached at about pollination time. Unfertilized ovules within ovaries containing developing embryos attained a 25 percent greater length than those in unpollinated pistils. This difference in length appeared April 22 (about fertilization time), and it was maintained throughout the remainder of the study (Figure 2). It is known that developing seeds are rich in auxins, gibberellins, and cytokinins, which control and regulate their growth and that of surrounding fruit tissue. The synthesis of some of these growth substances is triggered by the presence of pollen tubes or by the fertilization process (Krugman, et al., 1974). Possibly, the production of growth-promoting substances in fertilized ovules slightly affects the growth of nearby ovules.

Approximately one week following apparent fertilization, initial ovule abortion occurred in unpollinated material; similar abortion also occurred in unfertilized ovules of pollinated material, but it was delayed about two days. Possibly, this delay is occasioned by factors responsible for the somewhat longer unfertilized ovules in pollinated material. This abortion consisted of a gradual collapsing and aggregation of the nucellus and gametophyte tissue inside the integuments. This tissue remained attached to (and shrank toward) the chalazal end of the ovule. The apparently harder, lignified integument maintained ovule size as that achieved with mature embryo sacs, throughout the remainder of the study.

During the last 10 days of the study, the aments and pistils of both pollinated and unpollinated material elongated slightly. Principal change, however, occurred within the pollinated ovules, These increased in length by 50 percent, to their apparent mature size, 1.4 mm, and the embryos changed from globose to oval to initial and eventually full differentiation

of cotyledons and radicle. This most advanced embryo studied was not yet mature, however, occupying less that 50 percent of the ovule and surrounded mostly by free-nuclear endosperm; cellular endosperm was forming.

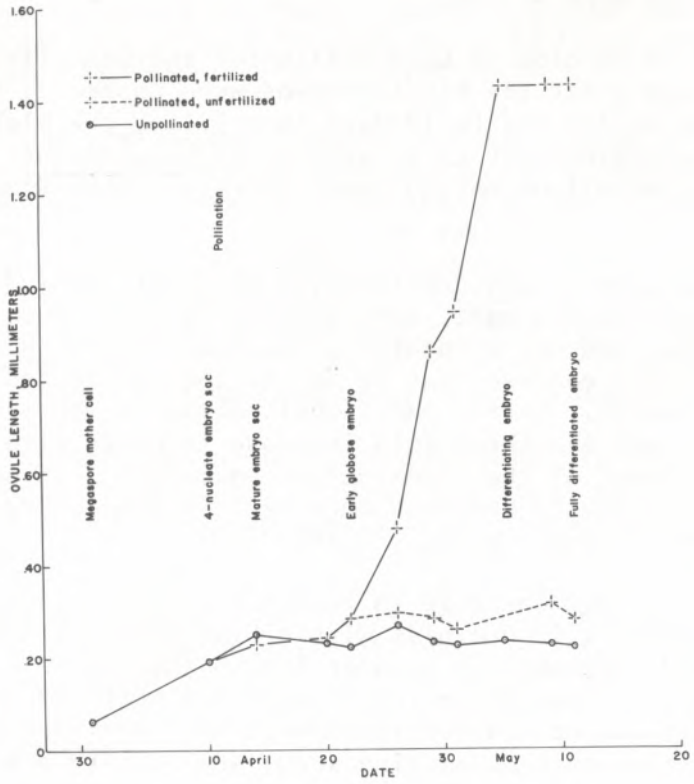

Fig. 2.--Ovule length of pollinated and unpollinated quaking aspen, 1973. Gametophyte and embryo stages identified are the most advanced stages observed on a given date.

Unpollinated ovules, and unfertilized ovules in pollinated ovaries, maintained approximately the same size as they had when abortion began (0.22 and 0.30 mm, respectively), but their internal degeneration became more complete.

Before the study was completed, leaf bud burst occurred in the following order, beginning on May 5: bagged, pollinated -> bagged, unpollinated unbagged.

There was no noticeable difference in abscission of aments between pollinated and unpollinated flowers.

SUMMARY AND CONCLUSIONS

The development of unpollinated and control-pollinated quaking aspen ovules was studied from the bud stage and to the fully extended female ament.

Both the pollinated and unpollinated ovules developed similarly from the megaspore mother cell stage to the mature female gametophyte stage with the fusion nucleus centrally located in the embryo sac.

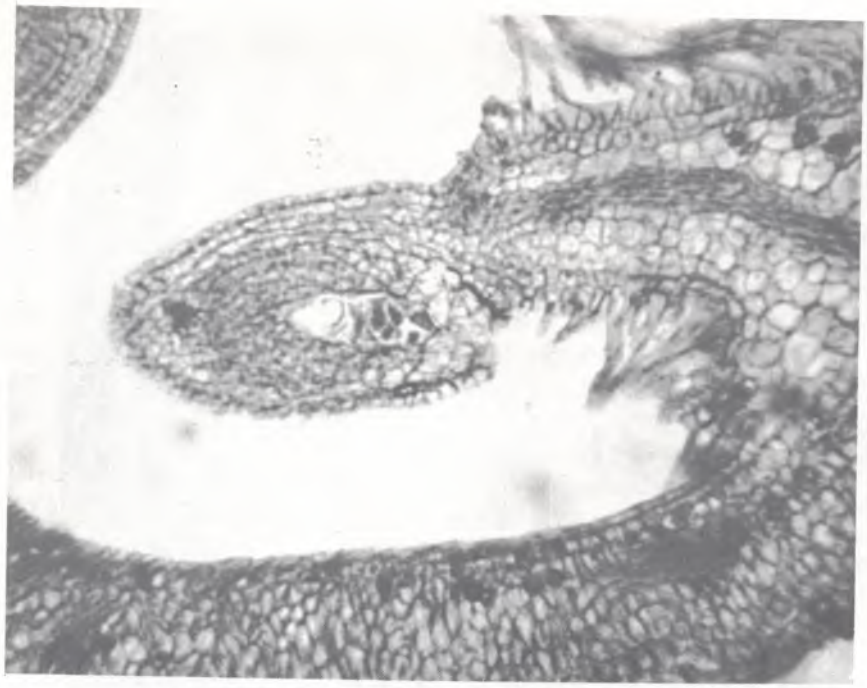

Figure 3.--Pollinated quaking aspen ovule a very few hours after fertilization, showing early embryonic development.

Figure 4.--Unfertilized ovule within ovary containing fertilized ovules about 10 days following the date on which fertilization would have occurred. These ovules reached about 25 percent greater length than unfertilized ovules in unpollinated pistils.

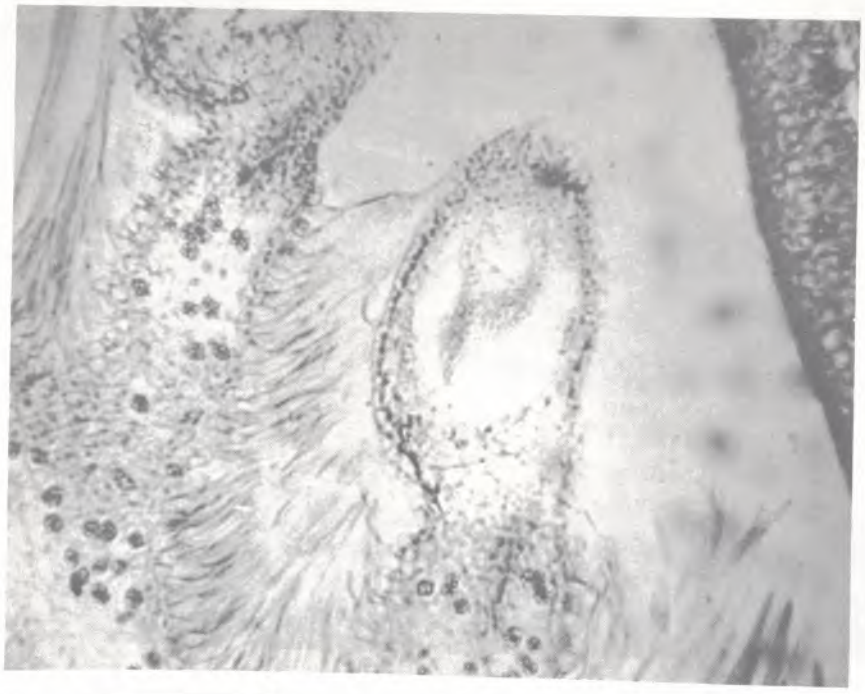

Figure 5.--Unpollinated quaking aspen ovule one week following normal fertilization date, showing typical abortion; aggregation and collapsing of the nucellus and embryo sac, attached to and shrinking toward the chalazal end of the ovule.

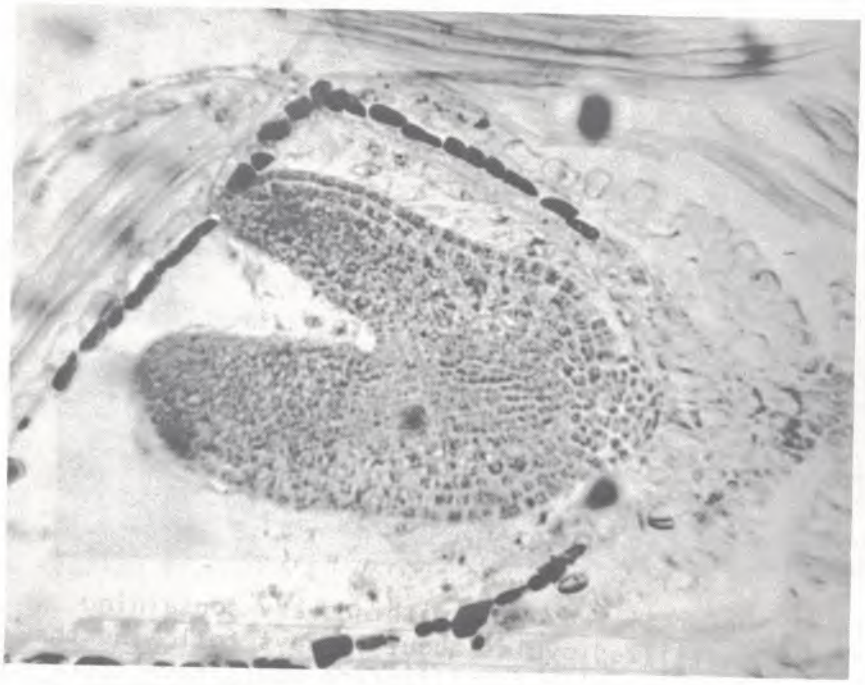

Figure 6.--Quaking aspen embryo approximately 20 days after fertilization.

From this stage forward, vivid differences occurred. Following fertilization, the aments, pistils, and ovules elongated rapidly, fully-differentiated embryos appearing within 20 days. In unpollinated material, the ovules began to abort about one week after fertilization would have occurred, and about two days later the same occurred in unfertilized ovules contained in pollinated ovaries. This abortion consisted of a shrinking of nucellus and female gametophyte tissue attached to the chalazal end of the ovule.

Hair cells developed in both pollinated and unpollinated ovules, and they reached a length equal to the ovary and stylar interiors.

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