# OBSERVATIONS ON FLOWERING IN THE ASPENS[1]

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Two aspects of the floral biology of the aspens have received much consideration in recent years. They are the forcing of dormant flower buds into early bloom to facilitate controlled pollination, and the description of hermaphroditism in species which are believed to be generally dioecious. In this paper I will present additional findings that contribute to a more complete picture of the floral biology of the aspens.

The flower buds of the aspens are typically borne in the axils of leaves in the current year's growth. The differentiation and development of flower buds of trembling aspen ( Populus tremuloides Michx.) were studied using longitudinal sections of axillary buds embedded in paraffin, Material was collected biweekly from one male, one female, and two hermaphroditic trees. It must be emphasized that the dates given for various stages of development are approximate and apply only to the New Haven area in 1960.

All buds, both axillary and terminal, sectioned from the first collection on May 28, 9 showed an undifferentiated, dome-shaped meristem be tween newly formed inner bud scales. By June 7, in axillary buds the meri stem had greatly elongated and bract primordia were being initiated later ally. Thus floral initiation was first visible in the first week of June. There were no apparent changes in cell size or shape associated with the lateral protuberances which were to become bracts. Initiation and development of floral parts proceeded from base to apex in a spiral so that by June 14, bracts had elongated considerably at the base of the inflorescence while near the apex, bract primordia were still being initiated.

[1] This is part of a comprehensive study on flowering in the aspens being carried out in partial fulfillment of the requirements for the Ph.D. degree.

At this time the first indications of the cellular differentiation leading to the formation of individual flowers were visible. In the axil of each bract an oblong group of angular cells formed. These cells rapidly multiplied and differentiated into the cup-shaped perianth which surrounds the stamens (androecium) in male flowers and the pistil (gynoecium) in female flowers of the aspens. There were no visible morphological differences between male and female flowers at this point.

By July 9, differentiation had proceeded to a stage where male and female flowers could be distinguished in longitudinal sections. By July 15, sex determination could be made directly by removal of the bud scales and examination under hand lens or dissecting microscope. Thus, for aspens bearing flower buds, determination of sex by means of floral anatomy is possible during a period of about nine months or from mid-July to mid April.

On July 23, ovules were visible within the pistil of female flowers and stamens were pronounced in the male. Sections of material collected on August 10 showed differentiation of stamens into anthers and filament in males, while increase in size was the only visible difference in female flowers.

Some development, particularly in the shape and color of female stigmatic tissue, occurs during the late summer, autumn, and winter months under natural environment. However, Seitz (1958) has been able to obtain presumably normal flowering of males in September by treatment of material from European aspen (Populus tremula L.) collected on August 15. This would suggest that in the aspens, by mid-August, floral differentiation and development has reached a condition where only the physiological requirement of cold treatment must be met before floral maturity can occur.

The second portion of this paper concerns the use of cold treatment in hastening early flowering in aspens. In 1930, Wettstein (1933) introduced the technique of forcing early flowering in Populus by placing the ends of cut branches with flower buds in water and keeping them in a warm environment. Under natural conditions the flower buds of aspens are physiologically ready for forcing by January, giving a period of about three months during which controlled pollinations can be made. This technique has been carried on by poplar breeders with few modifications. The following experiment was conducted to examine the possible improvement to the classical method.

Branches bearing flower buds were out from both male and female trees of bogtooth aspen (Populus grandidentata Michx.) at monthly intervals from October to January. The collected material was divided between two treatments, a thirty day cold storage at about +5° C and a control treatment in the greenhouse. Each treatment was subdivided into a light regime of natural day length (decreasing) and a light regime closely approximating the increasing day length from January to April when aspen buds are kept dormant by a climate unfavorable to flowering. Supplementary light for increasing day length was supplied by an incandescent bulb furnishing a minimum of 10 foot candles to all buds. The results are summarized in Table 1.

The number of days until flowering is presented only to indicate the insignificant effect of light on forcing flower buds of aspens. Where both males and females were successfully forced, the numbe rs represent an average number of days for males and females combined. As male flower buds opened one to two weeks earlier than females in the forced material, this average is not a good indication of the length of time to be expected before flowering.

Table I shows that cold storage hastens early flowering and that male flower buds can be forced at an earlier date than females given the same treatment Pollinations using the flowers forced in this study produced seedlings according to the schedule in table 2.

Table 1.--Summary of forcing data

| Collection date | 30 day cold storage | | Greenhouse | |
|---|---|---|---|---|
| | Simulated day | Natural day | Simulated day | Natural day |
| October 2 | ♂ only 18 days | ♂ only 18 days | No flowering | --- |
| October 31 | ♂ and ♀ 24 days | ♂ and ♀ 29 days | No flowering | --- |
| November 26 | ♂ and ♀ 12 days | ♂ and ♀ 12 days | ♂ only 26 days | ♂ only 24 days |
| January 2 | ----- | ----- | ♂ and ♀ 15 days | ♂ and ♀ 15 days |

Table 2.--Schedule of seedling production from flowers forced by cold storage

| Item | Male | Female |
|---|---|---|
| Branch collection | October 2 | October 31 |
| Treatment | Cold storage | Cold storage |
| Placed in greenhouse | November 1 | November 26 |
| Pollen collected | November 20 | --- |
| Pollination | December 28 | |
| Seed collected | January 21 | |
| Seed germinated | January 26 | |

Thus cold storage can be used to extend by about one month the period during which controlled pollinations can be made with aspens. This simple modification of forcing technique has the added advantage that material for forcing may be considerably more accessible in October than in January.

Now that we have followed the aspen flower to maturity, certain interesting abnormalities are most easily observed in the blooming catkin. One abnormality is the terminal inflorescence. Terminal inflorescences were noted on two grafted scions from a tree bearing hermaphroditic catkins. In normal catkins at maturity, the bracts are dead appendages. In terminal inflorescences some bracts became leaf-like and showed considerable growth in addition to the formation of chlorophyll. These green bracts subtended functional flowers and viable seed was obtained by pollination of receptive female flowers in a terminal inflorescence. One terminal inflorescence slowly elongated and exposed female stigmatic tissue for a period of 3 1/2 months. Other terminal inflorescences elongated and developed green bracts for two months before dying at the apex.

A more commonly noted abnormality is the occurrence of stamens in a predominantly female catkin or pistils in a predominantly male catkin. Many reports of such hermaphroditism have been made in trembling aspen and European aspen. Although the frequency of trees showing hermaphroditism is believed to be low, sampling to determine this frequency is a difficult problem as the numbers of hermaphroditic flowers or catkins on any tree may be small. Observation of all catkins on twenty-five branches from a tree presumed to be male revealed only six hermaphroditic catkins on two branches.

Aside from the role of floral evolution in the current evolutionary position of the species, the significance of hermaphroditism may lie in the products of self fertilized flowers. Although the female stigmatic tissue usually passes maturity before pollen is released in hermaphroditic catkins, some selfing does occur naturally. Of 381 seedlings germinated from selfed hermaphroditic catkins of one tree, 80 exhibited a marked chlorophyll deficiency while the others seemed normal.

In summary, (1) floral initiation in trembling aspen occurs during late May or early June in the New Haven area, Differentiation and development of flowers of both sexes is rapid. By mid-July differentiation is sufficient for determination of sex in flower buds.

(2) A preliminary cold treatment for material to be forced into early flowering was used to obtain seedlings about one month sooner than by the classical technique.

(3) The lability of parts of the inflorescence in trembling aspen is indicated by the green, leafy bracts of terminal inflorescences and the variable expression of sex in some trees.

(4) Hermaphroditic catkins of trembling aspen are self compatible and a chlorophyll deficiency was noted among selfed progeny.

## Literature Cited

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