MICROSPOROGENESIS AND MEGASPOROGENESIS IN TSUGA CANADENSIS[1]

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In 1954 a study of the development of male and female flowers in Eastern hemlock (Tsuga canadensis) was started at Yale School of Forestry. During the investigation an attempt was made to obtain a pictorial, chrono logical record of the formation of pollen grains from microspore mother cells, and the ovarian development from differentiation up to the formation of archegonia ready for fertilization. Fresh material was brought into the laboratory at weekly intervals until the buds were well advanced, than at semi-weekly and finally daily intervals in order to follow the stages of meiosis and pollen maturation. The female buds and early male buds were fixed in a formalin-acetic acid-alcohol mixture, embedded in paraffin, and permanent microscope slides were prepared. Propriono-carmine smears of microsporangia were made from each periodic collection of fresh material, and all of the stages of meiosis, or reduction division, were photographed.

Microsporogenesis

In Tsuga canadensis the male, or microsporangiate, strobili appear as small buds a ong the axes of lateral shoots near the tips. Each strobilus consists of a cluster of microsporophylls which contain microsporangia (or pollen sacs) filled with microspore mother cells in the early stages, and with developing pollen grains after meiosis has occurred. The microsporangia walls consist of several layers of cells, the innermost of which is a tapetal layer (layer of nutritive tissue surrounding microspore mother cells). Tapetal cells disorganize as microspores mature.

Meiosis begins in late March or early April in the New Haven, Connecti- cut, area, and proceeds rapidly after the initial division has started. Each microspore mother cell undergoes the first series of stages (prophase, meta -phase, anaphase and telophase) within a period of thirty to forty-eight hours. There follows an interphase which does not consist of two cells, but apparent- ly of one cell with two nuclei and no cross wall. Almost immediately after the appearance of the two nuclei, each with n chromosomes, the second series of divisions begins. This series is mitotic in that there is no further reduction in chromosome number, and each of the twelve chromosomes divides longitudinally. At the end of the second series there are four separate microspores, each with one-half the somatic or diploid chromosome number of 24.

Following meiosis there is a period of development which lasts about fur weeks. During this time the microspores develop into mature pollen grains, growing in size and assuming flattened-sphere shapes. The microsporangia

[1] This study was made as a part of the very extensive investigation of Tsuga canadensis currently being undertaken by the Connecticut Agricultural Experiment Station. It is expected that publication of their complete results will provide information useful in the study of other species, and will greatly increase the value of Eastern hemlock in the Northeast.

enlarge and emerge from the bud scales into full light, changing in color from green to yellow. They dehisce about the first of May, and pollen is shed for several days. It was observed that pollen was shed from the southwest portion of the trees about twenty-four to thirty hours earlier than from the northern parts, with the maturation advancing toward the north around both sides of the trees. It is believed that the microclimate is the most important factor in the observed lag in pollen production. The effect of solar radiation is, felt to a greater extent on the sides of the trees on which pollen is produced earlier, resulting in earlier drying and dehiscing of the microsporangia walls.

Megasporogenesis

Megasporangiate (or female) strobili are formed on the tips of lateral shoots in most instances , and in the earlier stages they appear similar to veetative buds. In early spring they are very pronounced, with characteristic shape and larger size than either the vegetative or male buds. Formation of megasporangia (or ovules) occurs during late March, and by the middle of April the megaspore mother cells make their appearance within the ovules. These cells are considerably larger than those surrounding, and they are characterized by dense cytoplasm and large nuclei.

As the megaspore mother cell enlarges, the ovule also increases in size. At this stage it consists of an oval mass of tissue (the nucellus or megasporangium) surrounded by a single integument, with a micropyle directed toward the base of the scale. At the opposite end, the tissue of the integument and nucellus is continuous with that of the scale, Shortly before pollination the megaspore mother cell undergoes two successive divisions and forms four megaspores. The first division is meiotic (or reduction), and the second is mitotic. One megaspore continues to enlarge at the expense of the nucellar tissue surrounding it, then it undergoes a nuclear division and forms archegonia which are later fertilized.

(The various developmental stages described above were illustrated by 39 color slides.)