"Pollination Drop" Time at the Arnold Arboretum

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Come spring, the hidden pollination processes of conifers can be seen with a hand lens.

Pollination is a critical event in the life of seed plants. Observant gardeners tend to realize that conifer pollen, unlike that of most garden flowers visited by insects, is dispersed by wind. They are generally unaware, however, of the array of processes that relate to the capture of conifer pollen by the ovules—the future seeds. In the Arnold Arboretum's rich collection of conifers, pollen capture generally occurs in spring. These events are subtle and unobtrusive and can scarcely compete with the simultaneous extravagance of azalea and lilac blossoms. However, with a little patience—and the use of a hand lens—the viewer can observe a process that turns out to be both beautiful and mysterious.

Gymnosperms, the group of seed plants to which conifers belong, are distinguished by possessing exposed (or "naked") ovules at the time of pollination. The apex of the conifer ovule (the *micropyle*) receives the pollen directly, usually by means of a drop of fluid (*pollination drop*). Angiosperms, or flowering plants, on the other hand, retain their ovules within a closed structure (the *carpel*), and the process of pollination does not involve a pollination drop.

Although the pollination-drop phenomenon is easily observed, remarkably few detailed studies have ever been made of it. A large living collection of conifers, such as that of the Arnold Arboretum, offers the ideal opportunity to obtain basic information with relatively simple tools.

Taxonomic Distribution of Pollination Drops

Conifers that are known to produce a pollination drop include all the species that have been studied in the families Cephalotaxaceae, Cupressaceae, Taxaceae, and Taxodiaceae. In the Pinaceae, only the genera *Picea* and *Pinus* produce a pollination drop (*Abies, Cedrus, Larix, Pseudotsuga,* and *Tsuga* do not). These genera have a range of ovular morphology and pollen-engulfing mechanisms that appear to be more specialized than the pollination drop and that are therefore thought to be derived, in evolutionary terms, from the drop system. Since non-coniferous gymnosperms like the cycads, *Ginkgo,* and *Ephedra* possess a pollination drop, it is usually assumed that this general mechanism is ancestral. There is even fossil evidence for the existence of a pollination drop (Rothwell,1977).

Recently, Professor P. B. Tomlinson of Harvard University has determined that most of the Podocarpaceae in the South Pacific possess a pollination drop associated with unusual pollen-retaining features. Other Southern Hemisphere conifers, namely *Agathis* and *Araucaria* (Araucariaceae), lack pollination drops and have developed unusual mechanisms involving long pollen tubes.

Observing Pollination Drops

In most conifers native to temperate latitudes, pollen release in spring is a clear indication

Juniperus communis. *Each cone produces three ovules and three droplets, one of which was removed for this picture. The droplets remain separate because the integument orifice of each ovule has an oblique orientation.*

that ovules are receptive. Conspicuous exceptions to this rule are *Cedrus*, which sheds its pollen in fall, and *Calocedrus*, which sheds its pollen in the winter. Table 1 lists the times when drops have been observed in various conifers in the Arnold Arboretum, as well as additional information about drops in natural habitats elsewhere in Massachusetts and Canada.

While most of the data in Table 1 was collected during daylight hours, it is known that secretion of the drop occurs at night in most *Pinus* species. It seems likely that nighttime initiation of the drop is a general phenomenon, while its persistence into the day is variable. Dr. John Owen at the University of Victoria in British Columbia has studied the conifers of western North America and discovered that individual ovules on the same tree can exhibit the drop for varying lengths of time, two to four days in *Pinus contorta* (Owens et al., 1981) or one to two weeks in *Pinus monticola* (Owens and Molder, 1977).

Anyone having access to native or cultivated conifers can easily see the pollination drop. The chief problem is the logistical one of simply being able to locate ovulate cones, given that in large trees they are generally located only on the upper branches of the tree. On most cultivated trees, however, one can usually find a low-hanging branch with a few accessible cones. Pollination drops in the Cupressaceae are the easiest to observe, in particular those of the shrubby junipers.

At pollination time, the ovulate cones are small and inconspicuous, but once located, they are seen to be attractive and often colored (either pink, yellowish, or green), and the drop can be spotted on the tip of the micropyle. If the drop is not visible on the tree, a cut branch kept in water and enclosed in a plastic bag will usually produce drops in a warm room. One needs to make repeated observations to date the event in a given species because the time period varies among individuals and at different latitudes and altitudes.

Exudation and Pollen Capture

An ovule consists of a *nucellus* enclosed by its coat or integument. Above the level of the nucellus, the integument forms a narrow passage, the micropyle, which leads to a cavity surrounding the nucellar apex, the pollen chamber. Subsequent events that relate to fertilization occur within the nucellus and eventually result in the formation of an embryo. The integument becomes the seed coat as development proceeds, but these events all occur after pollination.

At the time of pollination, the ovule takes the form of a vase with a short neck, the mouth of the vase being the opening of the micropyle. This mouth may be either bi-lobed or, more often, irregularly lobed. Secretion of the pollination drop is thought to be the result of changes in cells at the apical part of the nucellus (Owens and Molder, 1980; Owens et al., 1981). Once produced, the fluid passes up the micropyle and makes a round droplet at its mouth. Examples of secreted drops are shown in the accompanying photographs.

If the integument has two apical lobes or arms, as in *Picea* and *Pinus*, the fluid may simply form a film between them, held by surface tension. In principle, wind-borne pollen has to fall directly on the pollen drop to be available for fertilization. Since this is a very small target, it is not surprising that a variety of mechanisms have been developed to make the process more efficient. Karl Niklas at Cornell University has evidence from wind-tunnel experiments that the aerodynamic design of the conifer cone produces wind eddies that cause pollen to fall out of the air currents (Niklas, 1985; Niklas and Pau U, 1982).

These physical mechanisms, however, do no more than deposit pollen in the general vicinity of the ovule. *Pinus* has apparently elaborated the mechanism of pollen capture by two further steps. First, the integument arms and surface of the ovule secrete microdroplets, only visible at high magnification, which can cause pollen to stick to the surfaces that produce them (Owens et al., 1981). Second,

Table 1. Pollination Drop Observation Times

TAXON	LOCATION	DATE
Cupressaceae		
Chamaecyparis obtusa	Arnold Arboretum	20 April, 5 May, 1989
C. pisifera	Arnold Arboretum	26 April 1989
*C. thyoides**	Gardner, Mass.	26 April 1989
Juniperus chinensis	Arnold Arboretum	3 April 1989
*J. communis**	Petersham, Mass.	28 May 1989
J. squamata	Arnold Arboretum	20 April, 5 May, 1989
*J. virginiana**	Concord, Mass.	26 April 1989
Thuja occidentalis	Arnold Arboretum	3 April 1989
T. plicata	Arnold Arboretum	3 April 1989
Pinaceae		
*Picea sitchensis**[1]	Sooke, B.C., Canada	late April 1978
*Pinus contorta**[2]	Victoria, B.C., Canada	May 4-20, 1980
Taxaceae		
Torreya nucifera	Arnold Arboretum	9 June 1989
Taxodiaceae		
Cryptomeria japonica	Arnold Arboretum	26 April 1989
Taxodium distichum	Arnold Arboretum	20 April 1989

* Natural populations; [1] Owens and Molder (1980); [2] Owens, et al. (1981)

Chamaecyparis thyoides *(White Cedar), scanning electron micrograph of young cone (x 240), collected April 20, 1989, at Cedar Swamp, Gardner, Massachusetts. The flask-shaped structures are ovules (young seeds) shortly before the time of pollination. Dissection and photography by Marcheterre Fluet.*

Juniperus virginiana. *Numerous pollination droplets terminate the female cones.*

Taxodium distichum. *Two ovules develop in the axil of each fertile bract. Arrow points to the droplet.*

the normal pollination drop secreted by the nucellar apex fills up the entire micropylar region and picks up any pollen adhering to the microdroplets. Withdrawal of the pollen drop by drying or even active absorption pulls adhering pollen down the micropylar tube and into the pollen chamber. Repetition of secretion and absorption can occur in unpollinated ovules in *Pinus*, but probably not in *Picea* (Owens and Molder, 1980).

In an experiment carried out in 1935, Doyle and O'Leary artificially added pollen to one of the two ovules of each bract of a pine cone. Where pollen was added, the drop was absorbed within five or ten minutes, while the fluid of an unpollinated ovule remained unchanged. The authors concluded that the presence of pollen is the stimulus for halting the secretion of the pollen drop.

In most conifers, after pollen is drawn into the micropyle, the integumentary arms, if present, collapse, and the micropyle itself closes by cell enlargement and division. The pollen chamber is thus sealed off, and pollen germinates within the closed cavity.

Whether the mechanism of pollen drop absorption is biological or physical remains unclear and only a few chemical analyses of drops have been made. The pollination drop of *Pinus nigra* contains three sugars—glucose, fructose, sucrose (McWilliam, 1958). In *Taxus baccata*, in addition to the same sugars, it contains phosphates, amino acids, peptide, and organic acids (Ziegler, 1959). A more recent study by Seridi and Chesnoy (1988) added an unidentified oligosaccharide that was consistently present in *Cephalotaxus*, *Taxus*, and *Thuja*. Additional amino acids and galacturonic acid are also present. Fructose seems to be the most abundant sugar, perhaps as a source of nutrition for the germinating pollen.

More Work Is Needed

Many uncertainties remain in our knowledge of pollination mechanisms in conifers. Doyle and O'Leary (1935) mention the differences between observations carried out in the field versus the laboratory. Ideally the subject should be examined in cones on attached branches, and an arboretum collection with

Torreya nucifera. *The enclosed ovule is exuding a droplet.*

some diversity of species can facilitate this work.

Pollination drops have great practical importance since they can be one of the factors that limit commercial seed production. Because seed supply often sets a limit to reforestation programs, pollination-drop research in British Columbia has been supported by the Canadian Forestry Service. At the same time, the academic biologist needs to know the evolutionary pathways by which alternative pollination mechanisms have been produced.

Spring is lilac time at the Arnold Arboretum, and the period when public visitation is at its peak. Those visitors who stray from the well-trodden paths will find their daring rewarded among the conifers. The pollination drop, when viewed with a hand lens, provides a window into a seldom seen world where beauty and biology come together in harmony.

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