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STRUCTURE AND COMPOSITION OF POLLEN GRAINS OF VERNAL PLANTS

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Among the first morphological studies of pollen were those of Malpighi (1686) and of Grew (1675). While numerous articles on their structure and composition of pollen have been contributed, the amount of data available in application to several problems involved in such study is yet somewhat fragmentary.

Some of the problems upon which pollen study has a bearing are: (1) Structural studies with reference to the taxonomic aspect; (2) the identity of pollen grains and content thereof in relation to hay fever; (3) the identity and composition of pollen grains used by bees for food, and (4) biotic studies relative to pollination. Among the contributions which have a taxonomic bearing are those of Von Mohl (1835), Smith (1876), Biourge (1892), Gilg (1895), Pope (1925) and Wodehouse (1926 and 1928). Dealing with the relation to hay fever are the papers of Sheppegrell (1922), Koessler and Durham (1926) and La Garde (1926). The articles of Sipe (1913) (unpublished thesis), Phillips (1924), Parker (1924) and Hoffman (1925) (unpublished thesis) pertain to the study of pollen grains as bee food. Very extensive is the literature dealing with biotic relationships. Among these may be mentioned the work of Sprengel (1793), Darwin (1876), Kerner and Oliver (1895), Pammel (1903) and Knuth (1906).

METHODS

In this study pollen grains from fifteen species of spring-flowering trees were included.

Microchemical Tests

For starch IKI was used. For sugars the Fluckinger reaction was employed and for proteins tests were made with eosin, Millon's solution and the Biuret reaction. Fats were tested with Sudan III.

Microscopic Studies

The pollen grains were secured fresh from the trees or preserved in an ice box. The grains were mounted and immediately measured in water with the high dry lens of a Bausch and Lomb microscope. Most of the camera lucida drawings were made with a Zeiss eyepiece of 15 x magnification. The relative size of the grains was drawn with low power and for detail of structure the 1.8 oil immersion was used. Acid nigrosin solution was used for staining pores and for differentiating their lids.

DESCRIPTION OF POLLEN GRAINS

Salicaceae

Salix longifolia Muhl. (Sand Bar Willow).

Shape sub-spherical; pores 3 without lids lying in midway in the germ sutures; surface finely reticulate between the germ sutures which appear smooth. Starch present in about 10 per cent of grains; trace of sugar; no reducing sugars.

Salix rostrata Richards. (Beaked Willow).

Shape oval to sub-spherical; size 19-27 u; pores 3 without lids located in germ sutures; surface finely reticulate except over germ sutures and pores which appear smooth. Starch present in 10 per cent of grains.

Populus deltoides Marsh. (Cottonwood).

Shape, biscuit; size 28-38 u; one large germ pore; surface dotted with fine pores which give the extine a somewhat tuberculate appearance; very little starch; decided Biuret reaction for protein.

Fam. Betulaceae.

Betula alba L. (White Birch).

Shape sub-spherical; size 28-34 u; pores 3-4 with plugs behaving like *B. papyrifera* when treated with acid nigrosin.

Betula alba var. *papyrifera* (Marsh.) Spoch (Paper Birch).

Shape sub-spherical; size 20-40 u; pores 3-4 rarely 5. The walls are distended around the pores, the intine being much thickened gives a bull's eye like appearance. When treated with acid nigrosin, the opening of the pore appears to be filled with a deep staining substance which swells and behaves more like a plug than a lid. The surface of extine is smooth. Starch is abundant in 95 per cent of grains.

Ostrya virginiana.

Shape triangular; size 25-33 u; pores 3 occasionally 4 — pores appear like those of *B. papyrifera* when treated with acid nigrosin; 95 per cent of grains contain starch.

Fam. Urticaceae.

Ulmus americana L. (White Elm).

Shape sub-spherical; size 27-40 u; pores 4-5 with lids; surface smooth; 95 percent of grains densely filled with starch; protein indicated by Biuret reaction.

Ulmus fulva Michx. (Slippery Elm).

Shape sub-spherical; size 27-35 u; pores 4-5 with lids; surface smooth; 80 per cent of grains dense with starch; dextrin present, trace of disaccharids; distinct protein reaction manifested by Biuret test.

Celtis occidentalis L. (Hackberry).

Shape sub-spherical; size 30-33 u; and 37-41 u; pores 3; surface smooth; 95 per cent of grains dense with starch.

Fam. Oleaceae.

Fraxinus americana L. (White Ash).

Biscuit shaped; size 24-32 u; pores 4-5; surface of extine smooth, inner wall perforated with reticulate depressions. Seventy-five per cent of grains containing starch; protein present.

Fraxinus pennsylvanica Marsh. (Red Ash).

Biscuit shaped; size 20-32 u, apparently two sizes 20-25 u and 25-32 u; pores 4-5, rarely 6; surface of extine smooth but perforated on inner surface by reticulate pores; 95 per cent grains contain starch; protein present according to Biuret reaction. Some of the pollen grains germinating before leaving the anther.

Fraxinus pennsylvanica var. *lanceolata* (Borkh.) Sarg. (Green Ash).

Biscuit shaped; size 20-32 u; pores 3-4 (common), rarely 5; surface of extine smooth, inner surface penetrated by reticulate pores; 95 per cent of grains contain starch; protein present. Pollen grains; some pollen grains germinating.

Fraxinus nigra Marsh. (Black Ash).

Biscuit shaped; size 24-36 u; pores 3, sometimes 4; surface of extine smooth, inner surface penetrated by reticulate pores; 95 per cent of grains contain starch; Biuret reaction and eosin test show protein. A few grains were germinating before shed by the anther.

Fam. Aceraceae.

Acer Negundo L. (Box Elder).

Shape sub-spherical; size 26-37 u; pores 3 with germ sutures; surface of extine smooth, inner surface reticulate porous; 95 per cent of grains contain starch; protein present.

Name of Plant	Reserve	Food			Structure			
	Starch	Sugar	Protein	Fat	Size in Microns	Shape	Pores	Surface
SALICACEAE								
Salix interior	10% grs.	Trace	Present	----	21-25U	Sub-spherical	3	Reticulate ridged
Salix rostrata	10% grs.	------	Present	----	19-27U	Oval sub-spherical	3	Reticulate ridged
Populus deltoides	95% grs.	------	Present	----	28-38U	Biscuit	One lg num. fine	Slightly tuberculate
BETULACEAE								
Betula papyrifera	95% grs.	------	------	----	20-40U	Sub-spherical	3-4 rarely 5	Smooth
Betula alba	95% grs.	------	------	----	28-34U	Sub-spherical	3-4	Smooth
Ostrya virginiana	95% grs.	------	------	----	25-33U	Triangular	3	Smooth
URTICACEAE								
Ulmus fulva	80% grs.	Dextrin. Disacch. trace	Present	----	27-35U	Sub-spherical	4-5 with lids	Smooth
Ulmus americana	95% grs.	------	Present	----	27-40U	Sub-spherical	4-5 with lids	Smooth
Celtis occidentalis	95% grs.	------	Present	----	30-33U	Sub-spherical	3 pores	Smooth
OLEACEAE								
Fraxinus americana	95% grs.	------	Present	----	20-36U	Biscuit	4-5	Smooth
F. pennsylvanica	95% grs.	------	Present	----	20-32U	Biscuit	4-5 rarely 6	Smooth
F. pennsylvanica var. lanceolata	95% grs.	------	Present	----	20-32U	Biscuit	3-4-5	Smooth
F. nigra	95% grs.	------	Present	----	24-36U	Biscuit	Usually	Smooth
ACERACEAE								
Acer negundo	90% grs.	------	Present	----	26-37U	Sub-spherical	3	Smooth
Acer. saccharum var. nigrum	90% grs.	------	Present	----	37-54U	Sub-spherical	3	Smooth

Acer saccharum var. *nigrum* (Michx. f.) Britton. (Black Maple).

Shape sub-spherical; size 37-54 u; pores 3; surface of extine smooth; starch present in 95 per cent of grains; protein present.

DISCUSSION

Of the trees considered, only those of the willows and of the hard maple appear to be visited by bees. Not only the pollen but also the nectar is collected. All of these trees have abundant pollen which is distributed by the wind.

Scheppegrell points out that the shape, size and nature of the surface of pollen grains has a bearing on their causal relationship to hay fever and asthma. Among the smallest of pollens which have a causal relation to hay fever is that of ragweed measuring 15 μ in diameter and that of corn measuring 80 μ in diameter. By the application of Stoke's law the velocities and rates of fall of pollen of various diameters may be calculated. This knowledge compared with the amount of pollen produced and the abundance of the plant in question will give some idea of which plants might be expected to figure as possible stimuli of hay fever provided that their pollen protoplasts cause a positive reaction.

It will be noted in the data included in our table that there is not great variation in the size of the pollen grains examined. Compared with other lists of pollen measurements they range as average in size. They are practically smooth and approximately spherical in shape which characters facilitate their distribution by wind. According to tests made by Scheppegrell with the anemophilometer for testing the rate of travel of pollen, it has been shown, for example, that pollen of the common ragweed having a diameter of 15 μ will travel one-half mile under the same wind velocity at which corn pollen with a diameter of 80 μ will travel only 43 feet. Since the ragweed pollen is small and the plants are abundant, it becomes a greater problem than pollens which are larger and abundant.

The study of the form and the structural features of pollen grains have been used in the identification of source plants of honeys which contain them. Phillips, however, points out that this is not yet demonstrated a dependable method for bees are able to obtain nectar from some flowers without coming in contact with the anthers and, hence, such nectar may not contain the pollen grains of this plant. Also, in extraction of honey, there is danger of pollen grains from species of plants blooming at different seasons of the year entering the honey during extraction. Whether there is any mixing in comb honey has not been determined.

The chemical composition and structure of the pollen protoplasts and of their cell walls makes their behavior, when subject to various media or reagents in nature or in the laboratory of common interest in its application to hay fever phenomena and to the subject of pollen as food for bees, as well as to the behaviour of the pollen grain in germination under various combinations of environmental stimuli such as temperature, presence of moisure, or chemical reagents which involves similar principles. In other words, the protoplast of the pollen grain is inactive both with reference to its capability of acting as an irritant in producing hay fever and in its availability as food for bees as long as it is confined within its walls. The protoplast may be freed from its detaining wall (1) by normal germination (2) by bursting of the wall due to swelling and expansion when exposed to moisture secretions (nasal) or reagents, and (3) by mechanical injury. It has been noted by Phillips that in addition to the empty (digested) pollen grains found in the digestive tracts of bees that some were undigested, the coats not having been penetrated.

SUMMARY

All of the 15 species of vernal flowering trees studied contained starch in abundance. The willows had the least, only tbout 10 per cent of the grains containing starch. In the other 13 species starch was prominent in 80-95 per cent of the grains. No fats were present. Sugar was noted in *Salix longifolia* and in *Ulmus fulva*. Protein was observed in all of the species except those of the Birch family.

In shape the grains were nearly all sub-spherical when examined in water. The surfaces of all were smooth except those of the Willow family, which had a slightly tuberculate surface.

The four species of ash had a few pollen grains which germinated before the anthers were open.

None of these pollens burst when immersed in water which might imply either that the osmotic pressure was low or that the walls were with difficulty permeable.

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