A Route for the Transfer of Materials Through the Pollen Grain Wall. By J. R. ROWLEY,*†
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In the course of a study of pollen wall development in the Gramineae two interesting relationships were noted: the locule wall and tapetal cells were bordered by spheroids which were similar chemically (resistant to acetolysis) and morphologically to the pollen exine. The pollen exine as well as the wall of the spheroids was perforated by submicroscopic channels.

In the mature anther the spheroids averaged $0.45\ \mu$ in diameter without spines and had a central cavity measuring $0.2\ \mu$ (Fig. 4). Their surface was covered uniformly with small spines of the same size and shape as the spines on the grass pollen exine. The spheroids were located between the nutritive surface of the locule and the pollen grains (Fig. 2). The only exception to this positioning is that they were sometimes seen between adjacent tapetal cells. Where tapetal cells extended into the locule as in Fig. 2, these bodies followed the tapetal margin into the locule.

During the early microspore stages when the exine was thin, the spheroids were small, they had only a thin outer spinose ring of a density like the microspore exine, and the central area had an intermediate electron density. The pollen wall developed rapidly prior to microspore mitosis, and the size of the spheroids and thickness of their exine-like wall also increased until by microspore mitosis their dimensions were maximal. At this stage the central cavity had a low density similar to the background methacrylate (Fig. 4).

When microspore development was most intensive (intermediate between telophase II and microspore mitosis), cytoplasmic strands could be followed from the tapetum directly to the exine or from the tapetum to the spheroids and from there to the exine. After the intine matrix was formed (prior to microspore mitosis) giving a transparent and contrast-abetting region inside the exine, cytoplasmic strands could be traced from the pollen protoplast through the intine (Fig. 4) and lacunae of the exine. In 1952 Dahl (1) re-

ported strands of dense material in the intine of *Tradescantia paludosa*.

Thus the spheroids can be considered as part of the connections between the male gametophyte and its supporting tissue. Their proximity is not, however, necessary for exine building since spheroids were not found between adjacent microspore walls (Fig. 2) nor on the microspore surface toward the locule interior, though exine development here equalled that in direct contact with the tapetum and the zone of the spheroids. The number of channels through the exine and density of cytoplasmic strands traversing the intine were also undiminished around the portion of the exine which apparently is bounded by the fluid central part of the locule.

Since the wall-building substrates or their precursors must be derived from the locule walls and the tapetum, the spheroids may be formed under the same conditions as storage bodies such as starch and calcium carbonate crystals; *i.e.* in areas of excess substrate production or concentration.

Possibly the most important aspect of the spheroid bodies is that their morphology, composition, and position outside of the pollen wall seem to touch upon the prime problem of pollen morphology; *i.e.*, the source of control over the specific ornamentation of the exine. The spheroids in the Gramineae have the same ornamentation as the exine. Thus far this is also known to be the case for *Degeneria* (Degeneriaceae) in which the exine is quite smooth as are the spheroids, and for *Cryptomeria* (Pinaceae) in which both the surface of the pollen and spheroid bodies are granulate. If further sampling confirms this relationship, then either the influence of the pollen protoplast over exine form extends outward for a considerable distance, or exine development is at least partly controlled from the outside.

Pores 25 to $45\ m\mu$ in diameter were found to traverse radially the pollen wall and wall of the spheroids in several species of grass. In the pollen exine submicroscopic channels were demonstrated by three different methods: The exine was rendered sufficiently transparent to be examined directly in surface view (Fig. 1). Openings at the pollen surface were recorded by carbon replicas of the pollen surface were recorded by carbon replicas of formvar impressions (Fig. 3). In sections, channels

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were seen after a variety of fixations in both methacrylate and "araldite" embedded materials. They were recorded perpendicular and parallel to the surface of the pollen grains both before and after removal of the embedding material followed by shadowing with metals (Fig. 5).

Channels in the exine measuring about 15 μ were demonstrated by B. M. Afzelius in *Corylus avellana* pollen (2 and 3). Small pits having a diameter of about 40 μ are observable in Bradley's (4) surface replicas of *Plantago lanceolata*. Thus, channels crossing the exine are not peculiar to the pollen grains of the grasses.

The cytoplasmic strands traversing the pollen wall can be considered as plasmodesmata. They probably should not be considered as examples of Schumacher's (5-7) ektodesmata for, though the pollen wall has a highly cutinized component and resembles the epidermal cell wall, pollen grains are not exposed to a gaseous environment until development is completed. Pollen grains are bounded by other pollen grains and tapetal protoplasm or fluid until their development is complete. Furthermore, as the wall reaches its mature form (between mi-

crospore mitosis and anther dehiscence) it has not been possible to demonstrate cytoplasmic strands in the intine.

This latter observation suggests that plasmodesmata are present only during active growth and that the pollen grain becomes more or less autonomous prior to being shed from the anther.

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EXPLANATION OF PLATE 256

FIG. 1. Electron micrograph made through the exine of *Phragmites communis* Trin. Pollen grains partly enveloped by a film of evaporated carbon were treated with concentrated HNO_3 . Isolated pieces of exine were obtained which were sufficiently transparent (after the technique of Bringmann and Kühn, 8) to allow direct examination. The bright spots are holes through the exine; the dark areas are raised portions bearing spines (black spots). Magnification, 15,000.

FIG. 2. Section of a *Poa annua* L. anther treated with amyl acetate to remove the methacrylate and shadowed with platinum. The locule wall (L) and tapetal cell (T) between the pollen grains are lined by spheroids. The light intine is traversed at this stage (premicrospore mitosis) by many cytoplasmic strands (compare Fig. 4). Single germinal pores, characteristic of grass pollen, face the locule wall at either margin of the figure. Magnification, 2,500.

FIG. 3. Negative carbon surface replica of *Zea mays* L. pollen. The pollen grains were cleaned in warm 10 per cent KOH, then dispersed and partially embedded in a formvar film softened with acetone, and finally mechanically scraped from the formvar surface (Bradley, 4). Following carbon evaporation the formvar was removed in concentrated HNO_3 (Moor, 9). The projections are exinous spines and the small dark pits the openings for the channels through the exine. Magnification, 13,000.

FIG. 4. Cross-section of a *Poa annua* L. anther with the locule wall (L) at the top, a row of spinose spheroids (S), and a pollen grain toward the bottom. Fixation was by phosphate-buffered 4 per cent formalin in 5 per cent sucrose followed by veronal-acetate buffered 1 per cent OsO_4 . Ethyl alcohol dehydration was followed by embedding in 4:1 butyl to methyl methacrylate. Strands of cytoplasm can be seen in the transparent intine (I) between the pollen cytoplasm and the exine. Magnification, 24,000.

FIG. 5. Cross-section of the exine (E) and some of the intine (I) of *Sorghum vulgare* Pers. pollen fixed with veronal-acetate buffered 1 per cent OsO_4 to which 0.02 gm. of sucrose per ml. was added (10). The material was dehydrated in acetone and embedded in araldite. Dense channels (c) can be traced through columellae (co) and along either side of lacunae (la). In this section the pores appear to terminate short of the intine due to the slightly oblique orientation of the section. Magnification, 44,000.