

from allergist working in the Mediterranean countries but also in Japan, California, Texas and Mexico where Taxaceae and *Juniperus* pollens should behave in like manner.

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Orbicules do not significantly contribute to the allergenic micro-aerosol emitted from birch trees

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Key words: *Betula verrucosa* 1; *Betula verrucosa* 7; immunogold electron microscopy; pollen; Ubisch bodies.

On the inside walls of the anthers of flowering plants, particles of respirable size (< 4 µm) may occur, which are called orbicules or Ubisch bodies (1) (Fig. 1A).

Orbicules are no valuable vectors for birch allergens.

If orbicules are dispersed into the atmosphere during the flowering season and are inhaled, they can easily penetrate into

the lower regions of the respiratory organs (1). If allergens are present in orbicules, they may therefore act as effective vectors of allergens (1). These hypotheses were tested in birch by investigating the formation of aerosol orbicules by scanning electron microscopy (SEM). The cellular localization of the major *Betula verrucosa* 1 (Bet v 1) and a minor birch allergen *Betula verrucosa* 7 (Bet v 7) was tested by immunogold

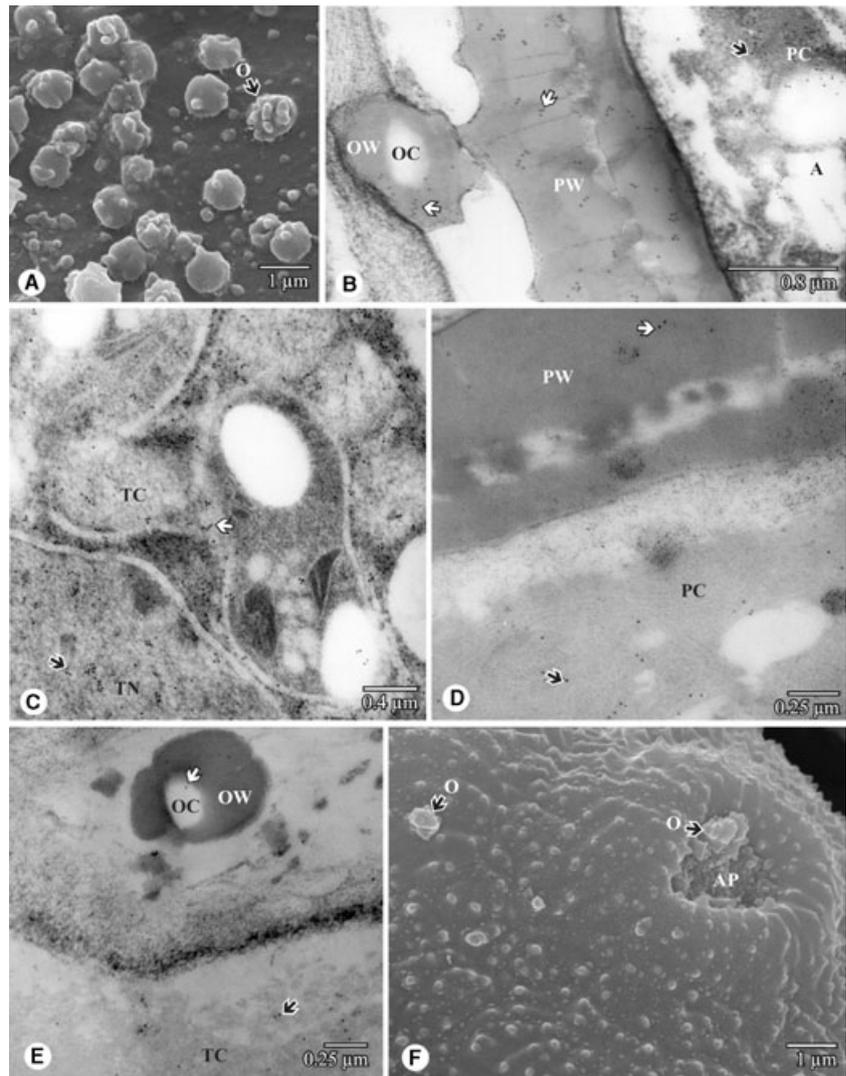


Figure 1. (A) Detailed observation of the inner wall of a birch anther covered with orbicules (O, black arrow). (B–C) Immunogold labeling results for *Bet v 7*. (B) Labeling within the orbicule wall (OW, white arrow), pollen wall (PW, white arrows), and pollen cytoplasm (PC, black arrow). No labeling is found within the orbicule core (OC) or the amyloplast (A) in the cytoplasm of the pollen grains. (C) Labeling within the cytoplasm (TC, white arrow) and nucleus (TN, black arrow) of a tapetal cell. (D–E) Immunogold labeling results for *Bet v 1*. (D) Labeling within pollen cytoplasm (black arrow) and only minor labeling in the pollen wall (white arrow). (E) Labeling is found within the tapetal cytoplasm (black arrow) and only minor labeling in the orbicule wall (white arrow). (F) Detailed observation of the pollen wall of an emitted birch pollen grain. One orbicule (O) is attached to the wall, another one to the pollen aperture (AP) (black arrows).

electron microscopy (IEM) across birch anther and pollen tissue.

Catkins from birch were harvested at early flowering and maintained in a controlled environment chamber. Stubs were placed below the flowers to capture particles emitted from the anthers during wind disturbance. The stubs were sputter coated and observed with a JSM-6360 SEM (JEOL Inc., Tokyo, Japan). For IEM, anthers were fixed (2.5% glutaraldehyde), and dehydrated (graded ethanol series), prior to embedding in Lowicryl K4M Resin. Ultra-thin sections were placed on gold grids and incubated with either primary polyclonal rabbit antiserum recognizing Bet v 7 (2) (1 : 200), or with primary monoclonal mouse antiserum against Bet v 1 (1 : 20) (mAb 5H8) (3), gift of R. van Ree, CLB, the Netherlands). Sections were incubated with gold conjugated (10 nm) secondary goat anti-rabbit or anti-mouse IgG (1 : 20) (Sigma-Aldrich Inc., St Louis, MO, USA), counterstained with uranyl acetate and lead citrate and observed in a Zeiss EM 900 (Zeiss, Oberkochen, Germany).

We localized the cyclophilin Bet v 7 in the pollen cytoplasm, pollen wall and orbicule wall (Fig. 1B). A comparable pollen cytoplasmic localization was recently reported for a low-molecular weight cyclophilin in different higher plants (4). Bet v 7 was also located within the cytoplasm of the cell layer bordering the pollen grains inside the anther (tape-tum cells) (Fig. 1C). In concordance with earlier reports, the major birch allergen Bet v 1 is shown to be predominantly located in the pollen cytoplasm (Fig. 1D). Only minor labeling was found in the pollen and orbicule walls of birch (Fig. 1D–E). Therefore, despite the homology between tapetum and sporogeneous tissue, from which orbicules and pollen grains originate respectively, we show that not all pollen allergens are found in orbicules.

We also investigated whether many orbicules are released from birch anthers. Our results indicate that only a small amount of orbicules (1–3/pollen grain) occurs free in the anther and can be dispersed (Fig. 1F). Therefore, as only few orbicules seem to be released from anthers of birch trees, and only minor labeling is found for the major allergen Bet v 1, we may conclude that orbicules do not significantly contribute to the allergenic micro-aerosol emitted from birch trees. However, this does not mean that the same applies to other species. For example, the major allergen of *Cryptomeria japonica* Cry j 1 was shown to be localized in the orbicules of *C. japonica*, *Cupressus arizonica* and *Cupressus sempervirens*, which are known to emit high numbers of orbicules during flowering (5).

It would be interesting to quantify atmospheric variations and clarify the timing of dispersal of different biologic micro-aerosols [parts of effete anthers, orbicules, pollen cytoplasmic fragments (6)] for each allergenic pollen source, in an attempt to establish correlations with clinical symptoms, and to estimate the different risks for patients sensitive to pollen allergens.

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