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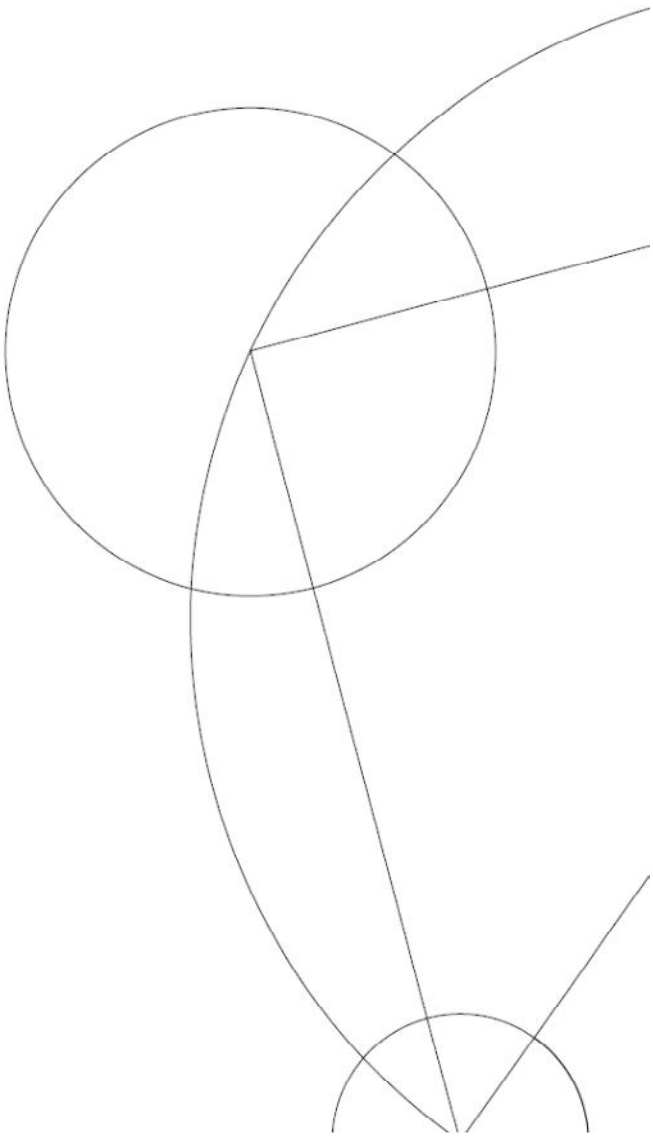

5.3. Development of *Podosphaera fusca* haustoria

Jesús Martínez-Cruz, David Vela-Corcía, Diego Romero and Alejandro Pérez-García

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC). Departamento de Microbiología, Universidad de Málaga. Bulevar Louis Pasteur 31, 29071 Málaga, Spain. E-mail: jesusmcruz@uma.es

The powdery mildews are obligate biotrophic pathogens that require living plant cells to complete its asexual life cycle. These pathogens develop a specialized structure responsible of nutrient uptake and factors exchange with the plant termed "haustorium". For this reason, these fungi establish an intimate relationship with the cytoplasm of the cells of its host. Despite being located inside the plant epidermal cells, the haustoria remains separated from the cytoplasm of the plant due to the plasma membrane of the plant, termed extrahaustorial membrane (EHM), whose differentiation and/or formation is still unknown. EHM is attached to the plasma membrane of the plant at a neck region termed septum. Between haustoria cell wall and EHM it is located the extrahaustorial matrix (EHMx), where the exchange of molecules between the pathogen and the host should take place. Within this matrix projections are extended from the haustorial body, termed haustorial lobes. In this work, we present a method developed for the isolation and purification of haustorial complexes of *Podosphaera fusca*, the main causal agent of cucurbit powdery mildew in Spain, from heavily infected zucchini cotyledons. The fungal cell wall was visualized by confocal microscopy with a specific antibody against chitin, one of the major structural components of the fungal cell wall. By this analysis, we could distinguish different structures like the haustorial body and lobes, neck and septum, but not EHM and EHMx. It was also observed that the size and number of lobes around the haustorial body increased progressively over time until completely surround the haustoria, which was indicative of the maturity of haustorial complexes. In parallel, we performed a detailed study of the ultrastructure of the haustorium by transmission electron microscopy (TEM). From the analysis of ultrathin sections of melon leaves heavily infected with the fungus, callose deposits were observed around the haustorial complex. Therefore, we conducted a double staining technique with aniline blue and calcofluor to display callose deposits. Thus, it was observed that callose deposits increased with time around haustorial complexes but without impeding the fungal development. Recently, it has been described the presence of small vesicles and multivesicular bodies (MVBs) in *Golovinomyces orontii* haustoria (Arabidopsis powdery mildew). Similarly, we performed a selective staining with a specific dye for plasma membranes in *P. fusca* haustoria, showing a different pattern in the distribution of small vesicles, endosomes and MVBs between haustorial lobes and body, and in addtion a high presence of large vacuoles in the haustorial body. Our results suggest that the lobes would be the responsible of vesicle trafficking and probably the main mediators of molecular dialogue with the plant.

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