

The Delivery of Cytoplasmic Effector in *P. infestans* Mediated by Secreted Exosomes

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Potato blight, a ravaging disease caused by the oomycete *Phytophthora infestans*, is a major threat to global food security. *P. infestans* secretes effector proteins that are delivered inside or outside plant cells to neutralise host immunity. Little is known about how and where effectors are delivered during infection. Here, two cytoplasmic effectors Pi04314 and Pi22926 C-terminally tagged a monomeric red fluorescent protein (mRFP) expressed in *P. infestans*. Confocal microscopy revealed that both effector fusions were secreted from haustoria, which form intimate interactions with plant cells, to accumulate at its sites of action in the host nucleus. The well-characterized apoplastic effector EPIC1 (a cysteine protease inhibitor), a pectinesterase (PE), a cell wall degrading enzyme, and INF4, a PAMP-like protein, was also secreted from haustoria. EPIC1, PE and INF4 secretion was inhibited by brefeldin A (BFA), demonstrating that they are delivered by conventional Golgi-mediated secretion. In contrast, the secretion of Pi04314 and Pi22926 was insensitive to BFA treatment, indicating that the cytoplasmic effector follows an alternative route for delivery into plant cells.

To further investigate the mechanism of the effector translocation from pathogen into host cells. Ultracentrifugation was performed to collect extracellular vesicles (EVs), which are secreted to facilitate intercellular and extracellular communication, to promote infection and evade host immune responses. These functions have been exploited by diverse organisms. Therefore, we hypothesised that EVs may play a key role in the dissemination of pathogen, also host-derived molecules during infection. Here, transmission electron microscopy has been performed to identify EVs, *in vitro* grown, *P. infestans*. Furthermore, immunoblotting shows that secreted EVs mediate cytoplasmic effector secretion but not for apoplastic effector. This is a major breakthrough in the plant pathology community.

Providing more details for helping to understand weapons of pathogen, to develop a specific chemical to disrupt these processes and inhibit pathogen infection.