Tackling Phytophthora

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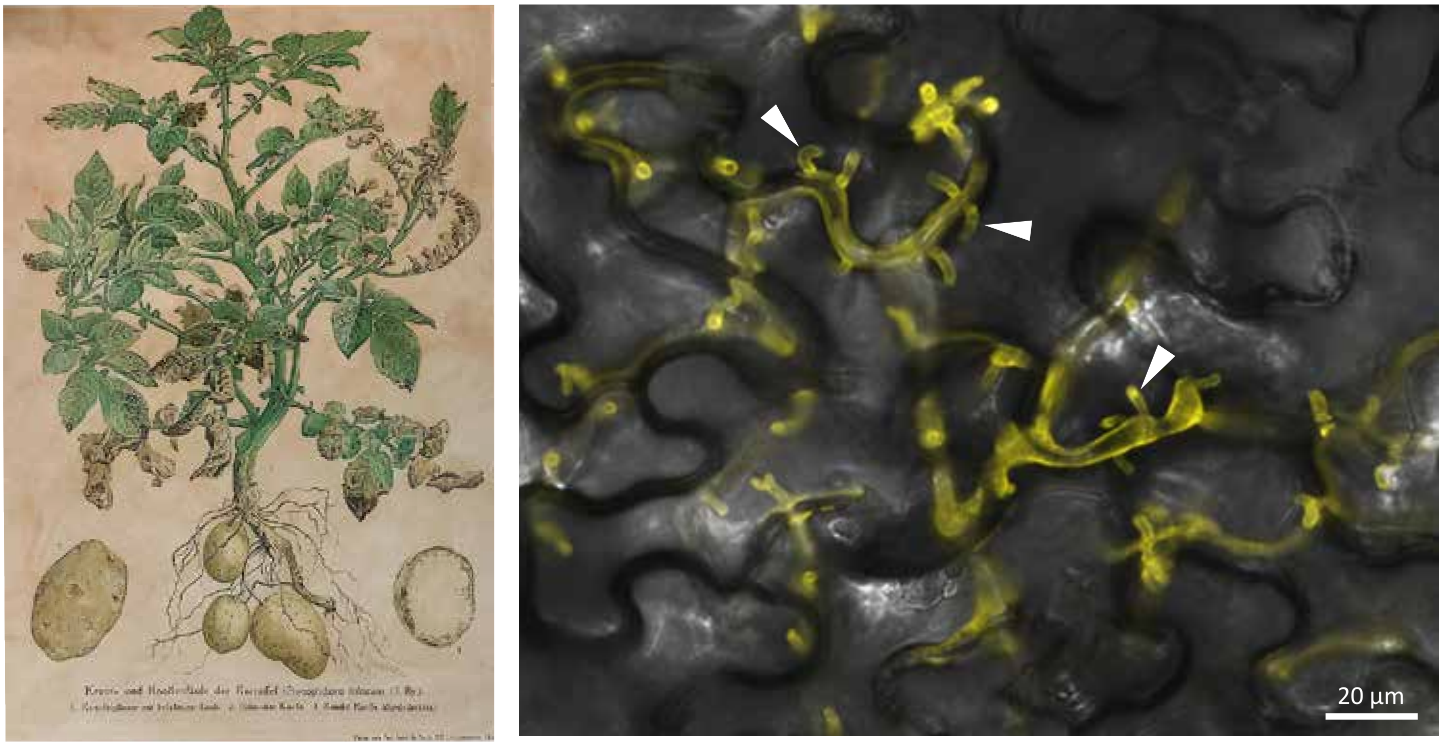
Imagine the Netherlands without potatoes: a horror scenario for growers and consumers, and affecting the processing industry and international trade. In the 19th century this scenario was close to reality when potato late blight raged across Western Europe. A turning point in history and an alarm bell for scientists to search for the cause of the disease. The microscopic villain was identified as a water mold that was named *Phytophthora infestans.* The oomycete genus *Phytophthora* (meaning plant destroyer) now comprises over 200 described species, the majority being destructive crop and forest pathogens. Here two researchers reflect on historic breakthroughs in *Phytophthora* research and describe how this research will be moving forward in the future.

The fact that potato is still on our menu, with the Netherlands as an important player in the global (seed) potato industry, implies that the fight against late blight was not lost. The main weapons are chemical control and breeding (Govers 2019). In the last decades many new resistance genes have been identified and thanks to advanced and novel breeding strategies and combined efforts in Public Private Partnerships, the arsenal of late blight resistant potato cultivars is growing (van der Vossen et al., 2007). This success is largely due to our increased understanding of the intimate interaction between *P. infestans* and potato, that enabled the exploitation of *Phytophthora* RXLR effectors to find new and more effective resistance genes (Govers, 2010; Rietman 2012; Vleeshouwers et al., 2008).

Tackling *Phytophthora* has been on the research agenda of the Laboratory of Phytopathology since its foundation in 1923, then named 'Laboratorium voor Mycologie en Aardappelonderzoek'. The first tracible publication with the title 'The saprophytic life of *Phytophthora* in the soil' was published by Helena L.G. de Bruijn in 1922 in 'Mededeelingen van de Landbouwhoogeschool' (24, paper 4, 40 pages) and the first PhD thesis on *P. infestans* defended in 1952 by Cornelis Mastenbroek, was entitled 'Over de differentiatie van *Phytophthora infestans* (Mont.) de Bary en de vererving van de resistentie van *Solanum demissum* Lindl.' His legacy is the once famous 'Mastenbroek differential set' that was widely used as international standard for *P. infestans* race identification. In five decades (1952- 2022) 32 PhD candidates graduated on *Phytophthora*



Drawing of a potato plant with typical late blight symptoms (left) and microscopic image of a leaf colonized by Phytophthora (right). After invasion the hyphae (depicted in yellow) proliferate in the intracellular space and form haustoria (arrowheads) that penetrate the plant cells.

Eduard Evangelisti (left) and Francine Govers showing cultures of their favourite pathogens, Phytophthora palmivora and Phytophthora infestans.

and/or oomycete research with a phytopathology professor as promotor, 22 of which were supervised by Francine Govers.

Francine: "When I joined the laboratory of Phytopathology in 1990, my predecessor Leen Davidse who was an expert in fungicide resistance (de Wit 2020), had just embarked upon a new research line on identifying pathogenicity factors in *P. infestans.* The discipline molecular plant-microbe interactions was in its infancy. For oomycetes there were no tools to tackle molecular and cellular processes and their genomes were a black box with only a handful of genes sequenced. The idea that we could rely on protocols and transformation vectors developed for fungi was too simplistic. The pioneering work by Howard Judelson (then at UC Davis), demonstrated that the choice of the promoter for driving expression of the selection marker is crucial for successful DNA transformation of *Phytophthora*. This must be an oomycete promoter re-emphasising that oomycetes are not fungi (Judelson *et al.*, 1991). We and others expanded the molecular toolbox. Major breakthroughs were the implementation of gene-silencing and more recently, CRISPR-Cas based gene-editing for functional gene analysis and live cell imaging allowing visualization of the dynamics of proteins and organelles at the subcellular level (Evangelisti et al., 2019; Kots, 2023). These advancements have revolutionised our approach to study plant pathogens, moving from mere observation – no matter how precise – to hypothesis testing and potential targeted treatments to combat diseases."

Unlocking the secrets of nature often feels akin to solving a complex puzzle. For oomycetes, a surprising revelation was the RXLR motif. This unique sequence of amino acids is present in hundreds of effectors that *Phytophthora* secretes as molecular weapons to manipulate plant cells to the invader's advantage. Unveiled during an annotation jamboree

(Govers & Gijzen, 2006; Govers & Meijer 2007; Seidl & Govers 2013) and supported by advances in genomics and international collaboration, this motif was subsequently identified as a crucial logistical signal for precise delivery of these weapons into plant cells. The devastating intimacy of oomycete-plant interactions just starts to be uncovered, with many secrets still awaiting discovery.

Francine: "In recent years we have established a successful collaboration with Joris Sprakel (WUR Biochemistry) and Tijs Ketelaar (WUR Cell Biology) to investigate the mechanisms underlying host invasion. By combining our expertise in physical chemistry, microscopy and phytopathology we could show that *P. infestans* does not rely on brute force to penetrate host cells but uses a more subtle slicing mechanism that we named 'naifu' (Japanese for knife) invasion (Bronkhorst et al., 2021, 2022). I anticipate that further unravelling of the cellular regulation and mechanobiology of naifu invasion will lead to clues how to tackle *Phytophthora* and eventually help in designing new strategies for crop protection. It is time for me to transfer the baton to the next generation, but I'm still eager to share my excitement for *Phytophthora* and contribute to inspiring discussions with the oomycete research group now spearheaded by Edouard Evangelisti."

Edouard: "I aim to unravel the cellular logistics that facilitates pathogenicity in *Phytophthora* and in a joint effort with Joris Sprakel and Tijs Ketelaar, want to decipher how oomycetes adhere to the host surface and subsequently infect plants. Building upon past discoveries and achievements, we have set an ambitious goal: constructing a digital twin of this destructive pathogen. This virtual model will integrate the main biochemical and biophysical cues that underlie plant infection to understand tricks and weaknesses of the pathogen. It will enable scientists to test how green tissue engineering can help combat the disease in an environmentally responsible manner. The quest for sustainable solutions is ongoing, fuelled by cutting-edge science and collective ingenuity."

The next time you have potatoes on the menu or savour a French fry, remember that behind these simple pleasures lies a complex and ongoing battle against microscopic villains fought with the sharpest tools of modern science. The fight against 'aardappelziekte' and its relatives is far from over. But with the promise of digital twins and the power of collaborative research, we're not just hoping for a brighter, late blight-free future; we're actively engineering it. Stay tuned!

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