

# Pest control

For **Dr Mary Wildermuth**, host-microbe interactions fire the imagination. With her multidisciplinary training and the development of innovative tools, she pinpoints the genes that govern disease and defence, using the resource-rich plant *Arabidopsis thaliana*

**What are the core objectives of your research? What inspired the creation of this study?**

I am fascinated by the intimate and intertwined nature of host-microbe interactions and what they reveal about shared biological control points and host biology. I define shared biological control points as host processes (or even specific proteins) targeted for manipulation by a variety of microbes. My research uncovers and defines these biological control points using the resource-rich plant *Arabidopsis thaliana* as the host organism.

Through my NSF-funded work, I am identifying *Arabidopsis* processes and components that mediate the sustained growth and reproduction of the powdery mildew (PM) *Golovinomyces orontii*. I find PM-host plant interactions especially interesting as PMs are obligate biotrophic fungi that acquire all nutrients from the living plant tissue through a localised feeding structure. Because the site of PM infection is highly localised, we developed laser microdissection techniques to isolate these plant cells for molecular analysis. By identifying genes (and the associated processes) that are specifically altered at the infection site, and assessing what happens when they are disrupted or overexpressed, we determine host biological control points of importance to the PM-plant interaction.

**What are the shortcomings of existing treatments and prevention? How will your research help to overcome such issues?**

Typically, deployed preventative and disease-responsive chemical applications (eg. sulphur, synthetic fungicides, and enhancers of basal resistance) are costly, and the timing of their application is critical. Because PM is not visible until the infection is well established, effective chemical control requires methods to predict and monitor PM onset. Meanwhile, classical and molecular genetic approaches have focused on plant genes that could



confer broad-spectrum resistance to PMs or enhance the plant's resistance to PM by increasing basal plant defence responses. Plant genes required by the PM fungus for its proliferation, referred to as compatibility factors, have received less attention.

In our research, we have identified and characterised plant genes that enhance basal disease resistance and facilitate PM growth and reproduction. Through the use of molecular breeding and/or GMOs that target sets of these genes, host PM resistance could be markedly enhanced. In particular, targeting sets of compatibility factors could result in more durable PM resistance than enhancing basal resistance (which acts against a variety of pathogens) or introducing a single resistance gene.

**How important is education and training to this work?**

The education and training of future scientists is critical to my mission as a Professor. Given my multidisciplinary background and research approaches, my laboratory includes members from a variety of scientific disciplines (plant biology, molecular and cellular biology, chemistry, and statistics) and I foster a collaborative lab culture in which members interact, thrive, and gain energy and insights from each other.

**Who have been the primary partners in this initiative?**

A key person on this project has been my postdoctoral researcher Dr Divya Chandran. She is excited by new technologies and has both excellent experimental and large dataset analysis skills. The project also benefited from a prior collaboration with Professor Terry Speed (UC Berkeley, Statistics). Speed was co-Principal Investigator on my previous NSF grant in which we jointly mentored graduate students and a postdoc in the development and application of new statistical methods to analyse temporally and spatially resolved *Arabidopsis* microarray data. Finally, my group benefits greatly from the publicly available and well-curated resources of the *Arabidopsis* community, and the willingness of colleagues such as Dr Masaki Ito (Nagoya University, Japan) and Dr Lieven De Veylder (Ghent University, Belgium) to share material and discuss findings.

**Could you outline the wider importance of this research?**

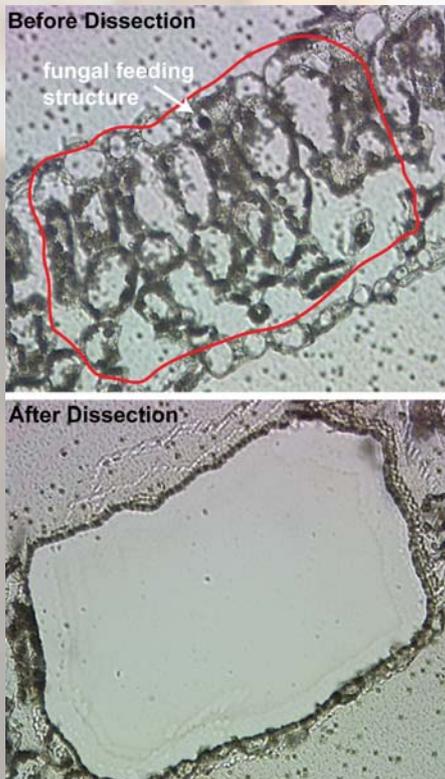
I see the wider importance of this work in layers. First, our findings can be translated to agronomic species to reduce PM-related crop loss, enhancing food availability worldwide. Second, a long-term goal is to integrate our information on specific regulators and processes impacting PM growth and reproduction into a mechanistic model to more accurately predict parasite response to changing environmental conditions or genetic modifications. Third, I anticipate some of our findings with the PM-plant interaction will apply to other microbe-host interactions, both in plants and mammals. Finally, I am motivated by the broader impact the study of host-microbe interactions can have on our understanding of fundamental host biology. Because pathogens such as PMs locally manipulate their plant host, there tends to be less genetic redundancy in the host response, which in turn allows individual components of a fundamental host process to be identified. This is certainly apparent in our use of the PM-interaction to investigate the cell cycle, and endoreduplication in particular.

# New ways to boost crop yield

Plant and microbial biologists at the **University of California Berkeley** at Berkeley show how cell-specific analyses can successfully identify novel targets to enhance disease resistance and promote plant growth

**POWDERY MILDEW (PM)** is a virulent microbe that attacks the outer epidermal cells in a wide variety of dicots and monocots including wheat, hops, roses, grapes and sugar beets. PM plagues farmers and floriculturists with its ability to spread through entire crops, thereby reducing product quality and yield. In order to address this problem, Dr Mary Wildermuth, Associate Professor of Plant and Microbial Biology at the University of California at Berkeley, and her team have been exploring PM effects on *Arabidopsis thaliana*, a small flowering plant used frequently in molecular biology to study plant-pathogen interactions.

*A. thaliana* becomes infected by PM when spores fall on a leaf. Given the right environmental conditions, the spore germinates, penetrates the leaf cuticle and forms a feeding structure within the epidermal cell. "Extensive surface growth of the fungus ensues, forming a hyphal network and asexual reproductive structures that contain chains of new spores. It is at this stage, five days



**FIGURE 1.** LMD OF CELLS AT PM INFECTION SITE FROM LEAF CROSS-SECTION

post-infection (dpi), that the PM is first visible to the naked eye," explains Wildermuth.

As an obligate biotroph that can only grow on living plant tissue, the PM fungus feeds off the nutrients in plant cells. To do so, it dramatically alters plant metabolism, eg. changing the infected leaf area from a carbon source to a carbon sink. In the process, photosynthesis is diminished and starch is broken down to supply the fungus with nutrients. Meanwhile the plant mounts limited defences to contain the infection. Taken together, this reallocation of plant resources can dramatically reduce product yield and quality.

The priority for Wildermuth's research group has therefore been to assess and characterise PM-altered genes and processes in order to determine host biological control points that may represent novel targets for enhanced PM resistance.

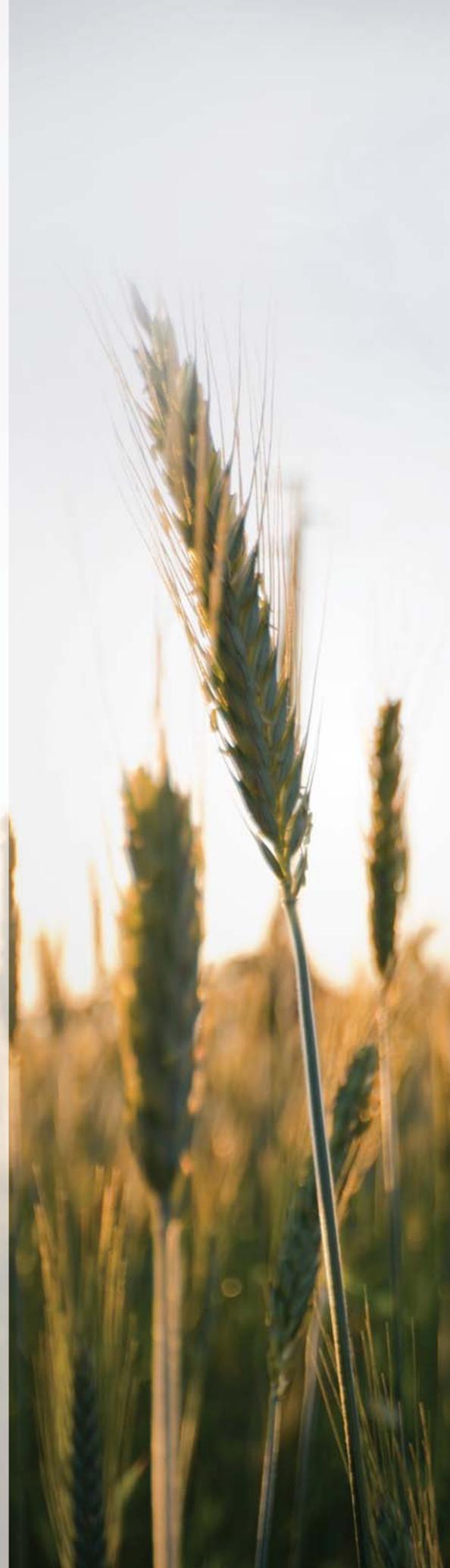
## NEW METHODS

To locate plant regulators and processes altered by the growth and reproduction of the powdery mildew on its host, the researchers employed laser microdissection (LMD) technology. Providing a higher resolution than whole leaf analysis, LMD allows the team to microscopically visualise the PM infection site and isolate specific cells for molecular analysis.

Thus far, Wildermuth's group has identified 67 plant transcription factors (TFs) with altered expression at the site of infection at five dpi: "Because TFs control expression of a suite of genes, their disruption is more likely to result in a detectable PM phenotype," she elucidates. Analysis and categorisation of mutants in these TFs is in progress. "Transcriptionally impacted processes include photosynthesis, carbon reallocation, respiration, cold/dehydration responses, defence, cell death, and the cell cycle," elucidates Wildermuth. "To date, we have had a very high pay-off rate, with a number of individual TF mutants resulting in PM phenotypes. In addition, because we perform process-based analyses, we can unveil PM phenotypes associated with multiple TFs acting in concert."

## ENDOREDUPPLICATION

Focusing at five dpi, Wildermuth then discovered an altered cell cycle known as endoreduplication that occurs in mesophyll cells underlying the fungal feeding structure. Endoreduplication



replicates cellular DNA but the cells do not divide, resulting in increased chromosomal DNA content (DNA ploidy). It is one of the few ways a cell can increase its metabolic capacity and size. And, in its absence, the UC Berkeley research revealed that PM proliferation suffers.

The team determined PM-induced endoreduplication is controlled by the transcription factor MYB3R4, a cell cycle regulator with induced expression at the PM infection site. "Since PM grows and reproduces less successfully when endoreduplication is blocked, our results suggest endoreduplication ramps up metabolism in cells that supply the fungus with nutrients," Wildermuth comments. "Targeting a set of PM compatibility factors (such as MYB3R4) can be advantageous for engineering or selecting for durable resistance."

Endoreduplication is not a new discovery, having been well documented in cells of plant and animal organs where increased cell size and/or metabolic capacity are beneficial, like corn kernels and tomato fruit. It has also been observed in response to other plant biotrophs that establish a sustained site of nutrient acquisition, such as root parasitism by nematodes and symbiotic interactions with Rhizobia. Wildermuth therefore considers locally induced endoreduplication a shared 'biological control point'. However, the mechanisms underlying its effect on metabolism have previously been unclear.

Delving deeper to assess the impact of ploidy on metabolism, Wildermuth analysed global expression data obtained from a number of plant systems in which ploidy and metabolism are enhanced. She found commonalities: "My analysis of cells with enhanced ploidy from different systems indicates that certain metabolic pathways (eg. fermentation) are specifically favoured when ploidy levels increase. In yeast, these pathways have been shown to confer a growth benefit when glucose is elevated, as we expect in the endoreduplicated cells".

With the relevant tools at her disposal, Wildermuth is confident she can explain how endoreduplication is induced and in turn how DNA ploidy affects metabolism: "We have now identified four *Arabidopsis* genes that impact mesophyll cell host ploidy at the infection site with a correlated impact on PM growth and reproduction. This indicates (induced) host ploidy acts as a determinant of PM susceptibility. We also recently identified an alternate metabolic pathway that operates at the PM infection

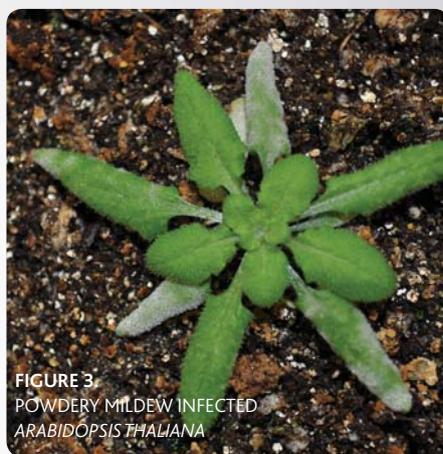


FIGURE 3. POWDERY MILDEW INFECTED ARABIDOPSIS THALIANA

site and is relevant to other systems with high metabolic demands." Wildermuth notes that novel factors influencing cellular ploidy and/or impacted metabolic pathways could potentially be used to increase phytochemical production, fruit, and seed size.

### IMPROVING RESISTANCE

Having determined components of the PM-host interaction, Wildermuth has begun to assess the candidates likely for improved resistance. In addition to PM compatibility factors such as MYB3R4 that can be disrupted to promote PM resistance by reducing the ability of the fungus to grow and reproduce on the plant, Wildermuth and her team have identified plant targets that enhance basal disease resistance against PMs and other biotrophic pathogens.

Plants synthesise the defence hormone salicylic acid (SA) in response to biotrophic pathogens to limit the extent of their infection. Although chemical treatment with SA or functional SA analogs has traditionally been used to protect against biotrophs, it is not without its limitations. Noticeably, SA can reduce yield and impact reproduction when used proactively. However if the plant's own ability to make SA is modified to provide a faster and heightened response to pathogen attack, these side effects could be limited. The Wildermuth group works on two plant targets in this regard: ICS1, the key SA biosynthetic enzyme, and PBS3, an important regulator of the SA pathway. Others have already translated Wildermuth's findings on ICS1 in *Arabidopsis* to agronomic species. PBS3 is conserved in plants such as rice and grapevine, making it, too, a promising target for enhancing disease resistance.

PM ranks as one of the most significant diseases impacting agriculture, and abating its growth is a constant struggle worldwide. By defining the processes and genes involved in the PM-host interaction with the goal of combining this knowledge to model disease outcome, the work of Wildermuth's investigation at the University of California Berkeley is setting a precedent for the future assessment and control of PM and other costly plant diseases.

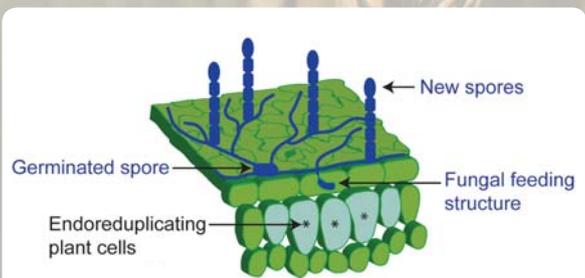


FIGURE 2. POWDERY MILDEW INFECTION SITE AT FIVE DPI

## INTELLIGENCE

### INFECTION SITE-SPECIFIC ANALYSES ELUCIDATE ARABIDOPSIS HOST PROCESSES AND COMPONENTS MEDIATING THE SUSTAINED GROWTH AND REPRODUCTION OF A COMPATIBLE OBLIGATE BIOTROPH

#### OBJECTIVES

- To develop an integrated understanding of the critical host responses controlling the extent of powdery mildew infection on *Arabidopsis thaliana*
- To characterise and classify infection site-specific plant transcriptional regulators based on their impact and function in the powdery mildew-*Arabidopsis* interaction
- To further define the plant factors responsible for fungal-induced increase in chromosomal DNA (ploidy) and the mechanisms by which increased ploidy facilitates powdery mildew growth and reproduction on *Arabidopsis*

#### FUNDING

National Science Foundation – Award No. 0958100

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**MARY WILDERMUTH** obtained a Chemical Engineering degree as an undergraduate at Cornell University. Her interest in biological questions led to her PhD in Biochemistry in 1997 from the University of Colorado, Boulder. Following postdoctoral research in the Department of Molecular Biology at Massachusetts General Hospital/ Department of Genetics at Harvard Medical School, she joined the Department of Plant and Microbial Biology at the University of California, Berkeley in 2003, where she is currently an Associate Professor.

