Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-40-5

Mosbach et *al*., High Throughput Imaging for Resistance Monitoring and Mode of Action Studies in *Botrytis cinerea* and Other Pathogens. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 239-240. © 2017 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-15-0

High Throughput Imaging for Resistance Monitoring and Mode of Action Studies in *Botrytis cinerea* and Other Pathogens

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INTRODUCTION

Fungicide resistance monitoring requires reliable, efficient and cost effective solutions to examine the spectrum of sensitivity within populations of pathogenic fungi. This usually entails sub-culturing of the pathogens originating from field samples. The sub-cultures are then tested on a range of fungicide concentrations based on mycelial growth or optical density. This method detects resistance effectively, but if the sub-culture is a bulk of resistant and sensitive strains, accurate determination of resistance frequencies requires molecular methods (e.g. pyrosequencing) or single spore isolations. Biotrophic fungi add another layer of complexity, because they require *in planta* testing or laborious microscopical evaluation. All of these processes are time and resource demanding and would benefit from an automated microscopical phenotyping. Here we describe how high throughput imaging with the Opera® High Content Screening System (PerkinElmer) enabled us to efficiently differentiate between SDHI sensitive and resistant fungal spores from an artificial bulk, and to do *in vitro* germination assays with a biotrophic plant pathogen.

FREQUENCY OF SDHI RESISTANCE IN BULKED *B. CINEREA* SAMPLES

Mixtures of *B. cinerea* spores of the sensitive reference isolate B05.10 and an SDHI resistant isolate (SdhB_P225L in the same genetic background) were prepared with varying proportions, based on counting with a haemocytometer. The spores were incubated in rich medium in 96 well plates in the presence of variable concentrations of three different SDHIs. After staining with Calcofluor White the cultures were imaged (4x objective lens), and the percentage of spore germination was quantified with a software developed in-house based on ImageJ, which allowed to differentiate between germlings and ungerminated spores. On average 370 objects were counted per spore bulk and treatment.

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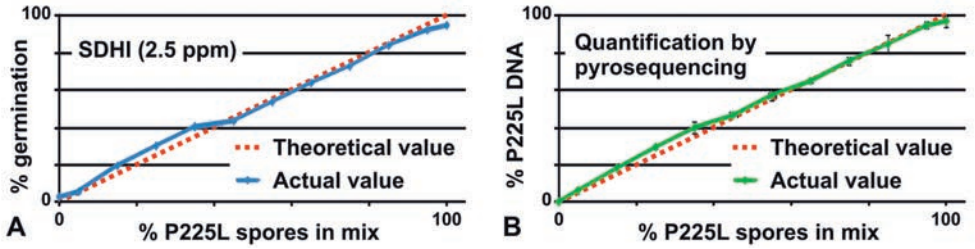

Figure 1 Correlation of the mean germination percentages at a given SDHI concentration (A) and the percentage of SdhB_P225L gDNA in the bulks as quantified by pyrosequencing (B) with theoretical values based on counting of the spores.

As shown in Figure 1, the average percentage of germination determined by image analysis at a particular SDHI concentration, which varied depending on the SDHI used (one example shown), correlated well with the expected values (spore counting with the haemocytometer).

IN VITRO SCREENING OF COMPOUNDS AGAINST *P. PACHYRHIZI*, THE CAUSAL AGENT OF ASIAN SOYBEAN RUST

Sensitivity assays based on the measurement of optical density are not applicable for the biotrophic *P. pachyrhizi*, but uredospore germination can be induced *in vitro* and is quantifiable via image analysis (Figure 2). This enabled us to screen for antifungal compounds that display a phenotype at early stages of development (e.g. respiration inhibitors).

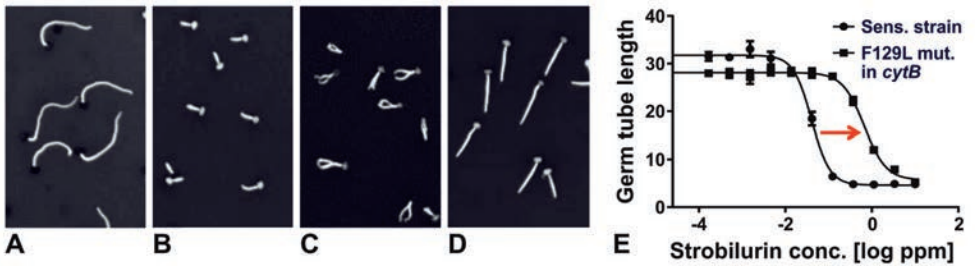

Figure 2 Phenotypical responses of *P. pachyrhizi* uredospore germlings to different compounds. A: Untreated control. B-D: Different compounds. E: Determination of sensitivity based on germ tube length. The tabular output of the automated image analysis was used to calculate EC_{50} values.

CONCLUSION AND OUTLOOK

Our equipment enabled us to do 96 well based spore germination imaging and analysis, allowing us to determine the proportion of resistant isolates in bulked samples and the screening of biotrophic pathogens. Preliminary imaging with sub-cellular labelling suggests that high content screening can be adapted to assist in the discovery of new mode of actions, making high throughput phenotyping a key asset in fungicide research.