Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-10-7

Hall S et *al.* Elucidation of a Novel Protein Kinase Target in Fungicide Research. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 85-86. © 2016 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-xx-x; URN

Elucidation of a Novel Protein Kinase Target in Fungicide Research

Hall S, Sangani D, Hansen R, Singh S, Earley F, Corran A

Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK
Email: samantha.hall@syngenta.com

Azaindole compounds, originating from a pharmaceutical lead in anti-inflammatory disease, were highlighted with broad spectrum fungicidal activity in Syngenta's *in-house* biology screens (Trejo et *al.* 2003). Our objective was to understand the mechanism of action of this chemical class in fungi relevant to agriculture.

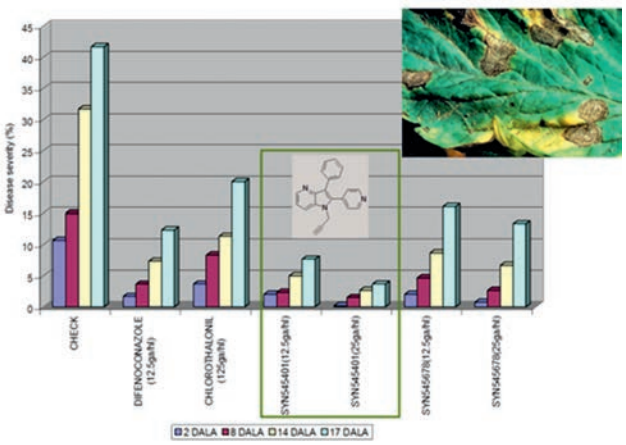

Figure 1 Efficacy of azaindole field candidates against *Alternaria solani* on tomatoes in Indonesia

Glasshouse activity was shown to translate to field performance against *Alternaria solani* (Figure 1) & *Phytophthora infestans*. Early assessment indicated inhibition of protein kinase enzymes might be responsible for the observed fungicidal effect. Subsequent protein kinase profiling screens revealed that the chemistry is selective, inhibiting only a small number of protein kinases.

Chemical proteomics strategies were developed for target identification in *Zymoseptoria tritici* and continue to emerge as attractive tools for probing ligand-protein interactions. Traditional affinity chromatography and innovative affinity-led purification (Figure 2) is being coupled to sensitive proteomic workflows that allow detection and quantification of native protein interactions within complex mixtures (Godl et *al.* 2003). A biochemical fractionation strategy was

employed to generate fractions where ligand binding profiles can be correlated with absolute and relative protein abundances as measured by quantitative MS^E workflows (Geromanos et *al.* 2009). Analysis and quantification of target enriched fractions by both chemical proteomics methods identified *Zymoseptoria tritici* HOG1 (OS2) kinase as a primary target of azaindole chemistry.

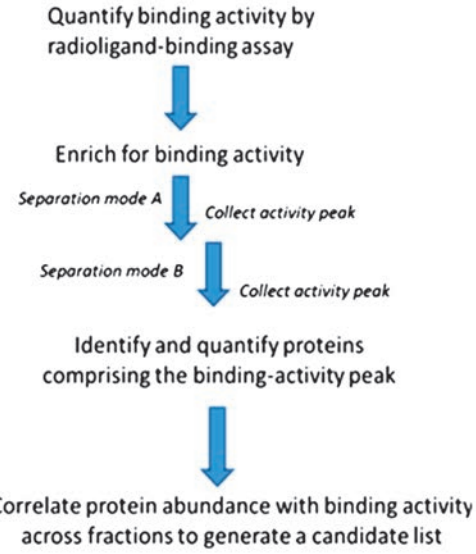

Figure 2 Target identification by affinity-guided proteomics

Magnaporthe grisea HOG1 and human p38-alpha gold standard kinase assays using ^{33}P radiolabelled adenosine triphosphate were developed as tools for structure activity determination to support chemical design.

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