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In Vitro Evolution of Fluxapyroxad Resistance in *Zymoseptoria Tritici*

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Zymoseptoria tritici is a highly adaptable plant pathogen. Due to resistance development to the methyl-benzimidazole carbamates (Griffin & Fisher 1985), the quinone-outside inhibitors (Fraaije et *al*. 2005) and some sterol-demethylation inhibitors (Clark 2006), chemical control of Septoria leaf blotch (SLB) has been marked by a continuous succession of fungicides with diverse modes of action. Options for sustainable disease control using optimal anti-resistance strategies are currently limited.

Recently, a new generation of carboxamide fungicides that inhibit the succinate dehydrogenase (Sdh) enzyme has been launched in the crop protection market. These new-generation SDHIs (e.g. bixafen, boscalid, fluxapyroxad, isopyrazam and penthiopyrad) are used in mixtures with azoles and/or multi-site inhibitors to reduce or delay fungicide resistance development in *Z. tritici* (HGCA 2014). However, mutational laboratory studies reported a number of Sdh subunit B, C and D target-site mutations in *Z. tritici* conferring a range of resistance levels to different SDHIs (Skinner et *al*. 1998, Fraaije et *al*. 2012, Scalliet et *al*. 2012). Monitoring studies conducted since 2003 have only detected four different *sdh* mutations in *Z. tritici* field isolates with low resistance factors to SDHIs since 2012. Isolates carrying these Sdh variants (B-N225T, C-T79N, C-W80S and C-N86S) were reported at low frequencies in France, Germany, Ireland and the UK, but control of SLB has not been affected so far (FRAC 2014). However, this might change as field strains carrying C-H152R, showing high resistance factors to SDHIs *in vitro*, have recently been detected in Ireland (Dooley et *al.* 2016) and the UK (Fraaije 2016). Insensitivity to SDHIs has evolved in *Z. tritici* field populations as predicted from mutational experiments.

In this study, we determined the *in vitro* evolution of resistance to fluxapyroxad in replicate populations of *Z. tritici* starting from the sensitive reference isolate IPO323. This isolate was exposed to increasing concentrations of fluxapyroxad in replicate populations at three different starting concentrations, each with or without exposure to UV light to increase mutation rate. After adaption to ten stepwise increases of the fungicide concentration, mutants carrying different *Sdh* mutations were found in most populations. One population without exposure to UV showed relatively low levels of SDHI insensitivity in the absence of target-site mutations.

Studies on archived populations over time using SNP detection Pyrosequencing assays showed that clonal replacement of Sdh variants (wt > C-T79I > C-H152R > C-S83G) occurred over time when the fungicide concentration increased (Figure 1).

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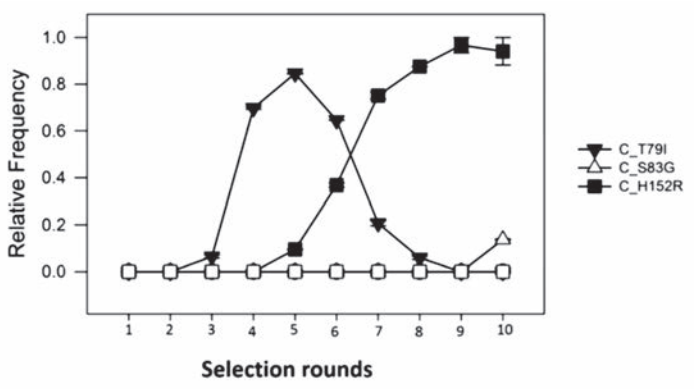

Figure 1 Allele frequencies representative of key SdhC amino acid substitutions in a fluxapyroxad-resistant UV-exposed population of IPO323 during ten rounds of selection *in vitro* on YPD amended with increasing (2-fold) concentrations of fluxapyroxad (0.08 to 40.96 µg ml^{-1}).

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