Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-29-9

Rehfus A et *al.*, Sensitivity of *Pyrenophora teres* to Succinate Dehydrogenase Inhibitors in Europe. In: Deising HB; Fraaije B; Mehl A; Oerke EC; Sierotzki H; Stammler G (Eds), "Modern Fungicides and Antifungal Compounds", Vol. VIII, pp. 175-180. © 2017 Deutsche Phytomedizinische Gesellschaft, Braunschweig, ISBN: 978-3-941261-15-0

Sensitivity of *Pyrenophora teres* to Succinate Dehydrogenase Inhibitors in Europe

Rehfus A, Strobel D, Bryson B, Stammler G

BASF SE, Agricultural Research Station, Speyererstr. 2, D-67117 Limburgerhof, Germany
Email: alexandra.rehfus@basf.com

INTRODUCTION

Net blotch caused by *Pyrenophora teres* is an economically important foliar disease in barley in all growing regions worldwide. The control of net blotch is mainly managed by the application of fungicides, including succinate dehydrogenase inhibitors (SDHIs).

SDHIs block the fungal respiration by binding to the ubiquinone reduction site of complex II also called succinate dehydrogenase (SDH), which is an essential enzyme in the mitochondrial respiration chain (Mathre, 1971, Matsson and Hederstedt 2001). The SDH enzyme consists of four subunits, the flavoprotein SDH-A, the iron sulphur protein SDH-B and two membrane anchoring subunits, SDH-C and SDH-D (Ackrell 2008). Cases of SDHI resistance in the field were mainly reported from plant pathogens on speciality crops such as *Botrytis cinerea* on grapes (Stammler et *al.* 2007) and *Alternaria alternata* (Avenot et *al.* 2009). Analysis of the *sdh* genes of those field isolates showed point mutations leading to amino acid exchanges in the subunits SDH-B, SDH-C and SDH-D e.g. B-H272Y/L/R in *B. cinerea* and C-H134R and D-H133R in *A. alternata*.

In 2012, first single SDHI resistant isolates of the cereal pathogens *P. teres* and *Zymoseptoria tritici* were found in Europe (www.frac.info). The emergence of SDHI resistance in *P. teres* in Europe was investigated in detail in a previous publication (Rehfus et *al*. 2016). In the following study additional information about the current situation is given, glasshouse tests and the development of combined resistance to quinone-outside inhibitors (QoIs) and SDHIs are shown.

MATERIAL AND METHODS

Sample collection and monitoring methods

To analyse the situation of SDHI resistance in *P. teres*, intensive monitoring programmes covering the main cereal growing regions in Europe were carried out for several years. Two different monitoring methods were used: 1) collection of spores from the air to generate single spore isolates (“isolate monitoring”) or 2) direct quantification of the causal point mutations

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-29-9

leading to SDHI resistance in leaf samples collected from trial sites by field technicians ("BASF field sampling monitoring").

The sampling of *P. teres* isolates was performed by EpiLogic (Freising-Weihenstephan, Germany). Spores from different European countries were collected in the air with a spore trap mounted on a car. In an *in vivo* assay the SDHI sensitivity of the isolates was tested (detailed methods are available online, www.frac.info). Isolates which showed ≥40% of necrotic leaf area at 0.64 mg L^{-1} fluxapyroxad were further analysed in our laboratory by molecular analysis of the *sdh* b, c and d genes, microtiter tests and glasshouse tests. Additionally, pyrosequencing assays for the quantification of point mutations were established to determine the levels of the resistance alleles in infected leaf samples collected at trial sites from all over Europe (detailed methods are described in Rehfus et *al.* 2016).

RESULTS

Situation of SDHI resistance in Europe

In 2012, the first two isolates of *P. teres* with sensitivities outside the baseline range to SDHIs were found in Northern Germany. Analysis of those isolates showed a target site mutation in *sdh* b gene leading to a histidine substitution to tyrosine at amino acid position 277 (B-H277Y). In 2013 and 2014, more resistant *P. teres* isolates were detected in Europe mainly in France and Germany ("isolate monitoring"). In addition to B-H277Y, four amino acid substitutions in SDH-C, namely C-N75S, C-G79R, C-H134R, C-S135R, and another five amino acid substitutions in SDH-D, namely D-D124N/E, D-H134R, D-D145G and D-E178K were identified. In France, 14% of the collected isolates in 2013 and 70% in 2014 carried an *sdh* mutation. In Germany, 44% of the isolates in 2013 and 47% in 2014 carried an *sdh* mutation. All isolates analysed carried only one single *sdh* mutation in one individual, never in combination with other *sdh* mutations. Most frequent amino acid substitution was found to be C-G79R, whereas other SDH changes occurred at lower frequencies. In Northern and Eastern countries such as Finland, Norway, Poland and Croatia, so far no resistant *P. teres* isolates were detected.

Also the net blotch infected leaf samples from trial sites analysed by pyrosequencing ("BASF field sampling monitoring") showed a similar pattern of resistance alleles in the years 2013 and 2014 (data not shown) as described before for the "isolate monitoring" of *P. teres*. The frequency of SDHI resistance alleles in *P. teres* infected leaf samples in 2015 is shown in Figure 1. In Northern and Eastern countries the population of *P. teres* was still totally sensitive towards SDHIs. In UK and Italy, in one sample of each country resistance alleles of up to 10% were observed. Highest frequencies of resistance alleles were detected in Northern France and Northern Germany with levels of up to 80% in some regions. In France, C-G79R amino acid substitution continued to have the highest frequency of all SDH alterations. In Germany, the situation was more heterogenous regarding the different resistance alleles, which could be also confirmed by the "isolate monitoring" 2015 (data not shown).

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-29-9

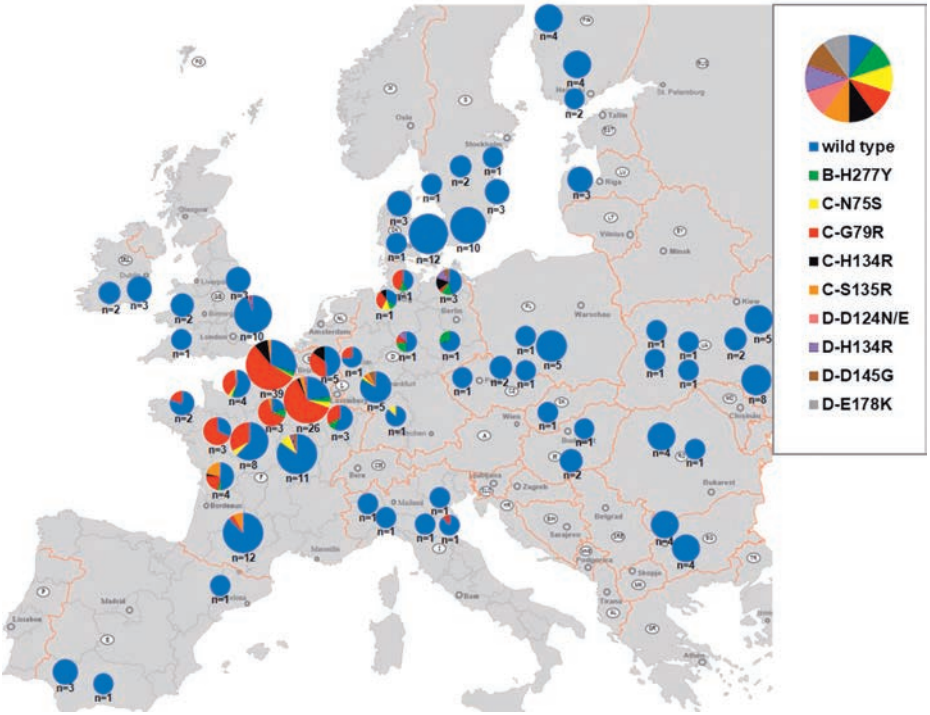

Figure 1 Frequency of amino acid substitutions leading to SDHI resistance in leaf samples with net blotch symptoms from various European trial sites in 2015 ("BASF field sampling monitoring", samples from untreated plots, n=260). The size of the disc represents the number of samples from different regions. Samples were analysed by pyrosequencing.

Impact of SDH amino acid substitutions in *P. teres* on the SDHI efficacy

Different SDH amino acid substitutions confer different levels of resistance to SDHIs. However, regarding each substitution seperately all SDHIs tested in microtiter tests are affected in a similar manner. In microtiter tests the highest EC_{50} values were obtained for isolates carrying mutations resulting in amino acid substitutions C-G79R and C-H134R followed by C-S135R and D-H134R. Medium levels of resistance were observed for isolates carrying C-N75S, D-D124E and low levels of resistance for isolates having B-H277Y, D-D145G and D-D124N.

The impact of SDH mutants on different SDHIs in greenhouse experiments was analysed. In Figure 2 the efficacies of three different commercially available SDHI solo fungicides (Fontelis®, Luna Privilege® and Xemium® with the active ingredients penthiopyrad, fluopyram and fluxapyroxad) on wild type isolates and SDHI resistant isolates of *P. teres* are shown. All three SDHIs solo compounds completely controlled wild type isolates of *P. teres* when applied one day preventative in full doses of the registered field rate (125 g ai ha^{-1}). Isolates carrying different SDH amino acid substitutions showed a broader range of inhibition levels depending

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-29-9

on the amino acid exchange and the product that was used. Regarding the different resistant phenotypes, the effect from the microtiter tests could be also observed in greenhouse studies. Isolates carrying amino acid substitution C-G79R, C-H134R had the highest impact on the SDHI efficacy, whereas B-H277Y, D-D145G and D-D124N had no significant or only a low impact on SDHI efficacies.

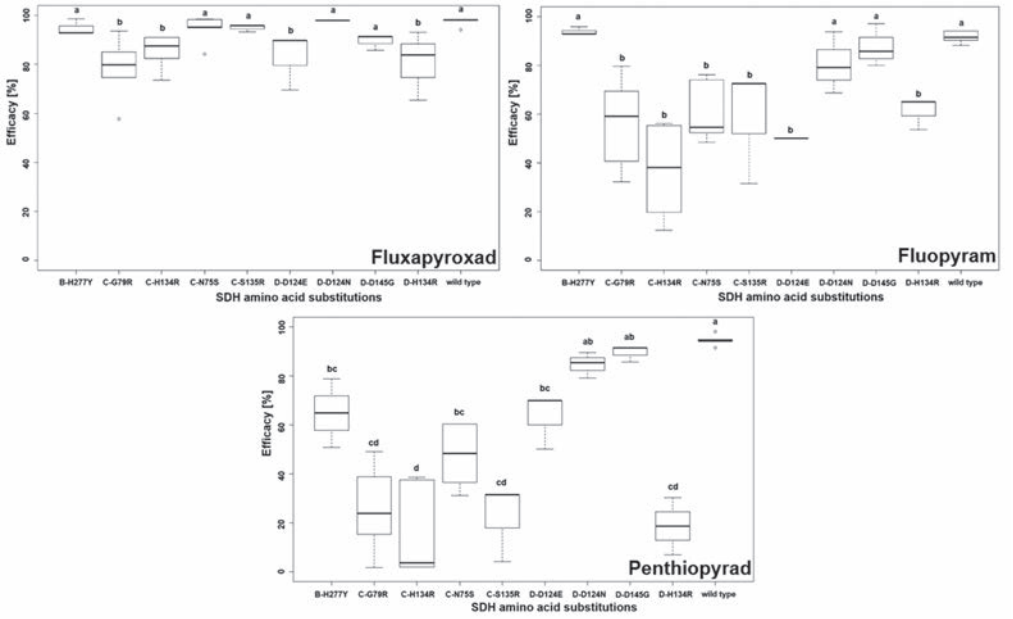

Figure 2 Efficacy of fluxapyroxad (Xemium®), fluopyram (Luna Privilege®) and penthiopyrad (Fontelis®) against wild type isolates and SDHI resistant isolates of *P. teres* in greenhouse experiments on barley cv. Astrid in one day preventative conditions. Dosage was 125.0 g ai ha^{-1}. Each treatment was repeated 3 times. Error bars were calculated from two isolates with three replicates, with the exception of mutations in *sdh* d. The Lagrange multiplier test was calculated with P=0.05, and significant differences in comparison with wild types are shown.

Fluxapyroxad showed a stable control with mean efficacies over 90% even for *P. teres* isolates with mutations leading to intermediate resistant phenotypes (e.g. C-N75S, C-S135R) in microtiter tests. A slight but significant decrease of fluxapyroxad efficacy was observed for *P. teres* isolates carrying the amino acid substitutions C-G79R, D-H134R, D-D124E and C-H134R with efficacies ranging from 78 to 86%. The compounds fluopyram and penthiopyrad were even more affected regarding most mutations.

Combined resistance to QoIs and SDHIs in *Pyrenophora teres* isolates

SDHI fungicides are often used in cereals in combination with QoI fungicides for a better and broader disease control. A higher tolerance to QoIs in *P. teres* is mainly mediated by the

Persistent Identifier: urn:nbn:de: 0294-sp-2017-Reinh-29-9

amino acid exchange F129L in cyt *b* of complex III. The frequency of F129L in *P. teres* populations in Europe varies between regions. However, F129L frequency was stable since many years within different regions (www.frac.info). In Germany for example a mean F129L frequency around 20% and in France around 30% was measured for at least three years (internal, unpublished data). Figure 3 shows the frequency of SDHI resistant isolates having F129L in combination analysed in the years 2013, 2014 and 2015.

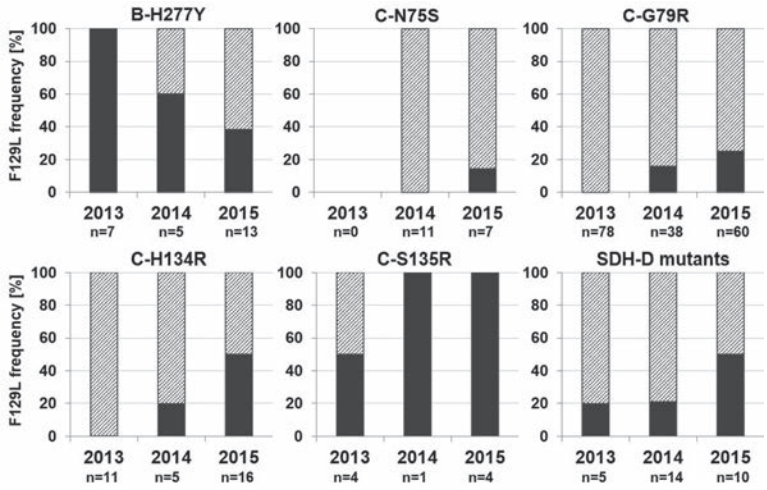

Figure 3 Frequency of F129L [%] (coloured in black) in analysed SDHI resistant isolates from European wide "isolate monitoring" 2013 to 2015. Isolates containing different SDH amino acid alterations are shown separately with exception of SDH-D mutants (D-D124N/E, D-H134R, D-D145G and D-E178K).

In 2013, only few isolates (mainly B-H277Y mutants) had the F129L exchange in combination to an *sdh* mutation. No isolate out of 78 C-G79R mutants showed the F129L exchange in combination. In 2014, the amount of double resistant isolates increased but the F129L frequency in SDHI resistant isolates was still significant lower than the frequency found in field populations (analysed by pyrosequencing from field samples, data not shown). However, in 2015 a further increase of combined resistance was observed in *P. teres* isolates with the exception of B-H277Y mutants, where a decrease of F129L in combination was detected.

CONCLUSION

In this paper, we report on the emerging situation of SDHI resistance in *P. teres* in Europe. High levels of resistance alleles were detected mainly in Northern France, and Northern Germany but lower frequencies were also observed in several other countries such as the United Kingdom. The pattern of resistance alleles varied between different regions and to

some extend from year to year. Greenhouse data show that SDHIs still contribute to disease control even in resistant isolates. Strict resistance management strategies are recommended to maintain SDHIs as effective tools for net blotch control. Efficient resistance management strategies are still available in net blotch as QoI fungicides still contribute to net blotch control (Semar et *al.* 2007) and the sensitivity to DMIs is stable over the last few years (www.frac.info).

REFERENCES

Ackrell BAC (2008). Progress in understanding structure–function relationships in respiratory chain complex II. *FEBS Letters* 466, 1-5.

Avenot H; Sellam A and Michailides T (2009). Characterization of mutations in the membrane-anchored subunits *Aa*SDHC and *Aa*SDHD of succinate dehydrogenase from *Alternaria alternata* isolates conferring field resistance to the fungicide boscalid. *Plant Pathology* 58, 1134-1143.

FRAC, Fungicide Resistance Action Committee. http://www.frac.info/ [accessed 07-08 2016].

Mathre DE (1971). Mode of action of oxathiin systemic fungicides. III. Effect on mitochondrial activities. *Pesticide Biochemistry and Physiology* 1, 216-224.

Matsson M and Hederstedt L (2001). The carboxin-binding site on *Paracoccus denitrificans* succinate:quinone reductase identified by mutations. *Journal of Bioenergetics and Biomembranes* 33, 99-105.

Rehfus A; Miessner S; Achenbach J; Strobel D; Bryson R and Stammler G (2016). Emergence of succinate dehydrogenase inhibitor resistance of *Pyrenophora teres* in Europe. *Pest Management Science* 72, 1977-1988.

Semar M; Strobel D; Koch A; Klappach K and Stammler G (2007). Field efficacy of pyraclostrobin against populations of *Pyrenophora teres* containing the F129L mutation in the cytochrome *b* gene / Feldwirkung von Pyraclostrobin gegen Populationen von *Pyrenophora teres* mit F129L Mutation im Cytochrom-*b*-Gen. *Journal of Plant Diseases and Protection* 114, 117-119.

Stammler G; Brix HD; Glättli A; Semar M and Schoefl U (2007). Biological properties of the carboxamide boscalid including recent studies on its mode of action. In: *Proceedings XVI International Plant Protection Congress Glasgow*, pp. 40-45.