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Sensitivity of Wheat Powdery Mildew (*Blumeria graminis* f.sp. *tritici*) towards Metrafenone

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Abstract A monitoring study on the current sensitivity situation of wheat powdery mildew (*Blumeria graminis* f.sp. *tritici*) towards metrafenone was performed in 2009 with 2509 isolates. Airborne isolates were randomly collected in different regions of the most important European cereal growing regions. The sensitivities of the majority of isolates were comparable to the baseline sensitivity, which was determined in 2000 before market launch of the compound. Ninety-two isolates showed sensitivities outside the baseline. Eighty-four of them were classified as moderately adapted and could still be inhibited with registered rates of metrafenone in glasshouse tests under various preventive or curative conditions. Eight isolates were identified to be resistant, which were not fully inhibited at registered rates. Resistance management strategies and further extensive monitoring studies are indicated for a sustainable use of metrafenone against wheat powdery mildew.

Keywords Metrafenone · Fungicide resistance · Baseline · Wheat powdery mildew · *Blumeria graminis* f.sp. *tritici*

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Sensitivität des Weizenmehltaus (*Blumeria graminis* f.sp. *tritici*) gegenüber Metrafenon

Zusammenfassung In 2009 wurden Monitoringstudien zur aktuellen Sensitivitätssituation des Weizenmehltaus (*Blumeria graminis* f.sp. *tritici*) gegenüber Metrafenon mit 2509 Einzelisolaten durchgeführt. Die Isolate wurden in verschiedenen Regionen der wichtigsten europäischen Getreideanbaugebiete mittels Sporenfalle gewonnen. Die Mehrheit der Isolate wies eine Sensitivität auf, die der der „Baseline“ (natürliche Wildtyp-Sensitivität) aus dem Jahre 2000 entspricht. Die „Baseline“ wurde vor der Markteinführung des Wirkstoffs erstellt. Die Sensitivitäten von 92 Isolaten waren außerhalb der „Baseline“. Davon wurden 84 Isolate als moderat adaptiert klassifiziert, welche mit registrierten Feldaufwandmengen im Gewächshaus unter verschiedenen präventiven oder kurativen Applikationsbedingungen sehr gut bekämpft werden konnten. Acht Isolate wurden als resistent eingestuft, welche mit registrierten Aufwandmengen nicht vollständig kontrolliert wurden. Für einen nachhaltigen und sicheren Einsatz von Metrafenon zur Bekämpfung von Weizenmehltau sind effektive Resistenzmanagement-Maßnahmen und auch ein intensives zukünftiges Sensitivitäts-Monitoring angezeigt.

Schlüsselwörter Metrafenon · Fungizid-Resistenz · Baseline · Weizenmehltau · *Blumeria graminis* f.sp. *tritici*

Introduction

Cereal powdery mildew (*Blumeria graminis* [DC.] Speer, formerly designated as *Erysiphe graminis* DC.) is an obligate biotrophic fungus and comprises as a species specialised physiological forms (*forma speciales*, f.sp.) based on

strict host specialisation such as *B. graminis* f.sp. *tritici* on wheat or *B. graminis* f.sp. *hordei* on barley (Wyand and Brown 2003; Olesen et al. 2003).

Metrafenone (3-bromo-2',3',4',6-tetramethoxy-2,6'-dimethylbenzophenone) is a fungicide registered for control of powdery mildews in different crops and eye spot (*Oculimacula* spp.) in cereals. It has a unique mode of action (Koehle et al. 2004; Schmitt et al. 2006). The biochemical mode of action is not fully elucidated, morphological studies showed that metrafenone reduced germination of spores, blocked appressoria formation and strongly reduced sporulation. Abnormalities in hyphal growth and malformation of conidiophores have been described under metrafenone exposure and most likely metrafenone acts by disturbing a pathway regulating organisation of the actin cytoskeleton (Opalski et al. 2006).

Cereal powdery mildews are classified by the Fungicide Resistance Action Committee (FRAC) as pathogens with high risk of developing resistance to fungicides (http://www.frac.info, pathogen risk list). Indeed, these pathogens have characteristics which support rapid adaptation such as their relatively short generation time, the production of large amounts of conidia over the whole season and their airborne spread. Wheat powdery mildew developed resistance towards different fungicidal modes of action in the past, such as benzimidazoles, QoI fungicides or the quinoline quinoxyfen (http://www.frac.info, list of resistant plant pathogenic organisms).

Therefore, extensive monitoring on the sensitivity of cereal powdery mildews towards metrafenone was indicated for a sustainable use of this fungicide. This paper describes the results of the European monitoring programme on wheat powdery mildew (*B. graminis* f.sp. *tritici*) and metrafenone in 2009 and glasshouse studies on isolates whose sensitivities were outside the baseline.

Material and Methods

Baseline Sensitivity—ED_{50} Determination

Studies on the baseline sensitivity were carried out in 2000 by EpiLogic (Freising, Germany) with 140 randomly trapped European isolates using a detached leaf method with the wheat variety "Kanzler" according to a previously published method (Felsenstein 1991; Felsenstein 1994; http://www.frac.info). A range of 9 test concentrations with active ingredient (a. i.) of 0, 0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16 mg/l, was used to determine the ED_{50} value of each isolate. Isolates from a total of 14 wheat growing regions in Ireland (1 region), UK (3), Belgium (1), France (3), Denmark (1), Germany (3), Austria (1) and Italy (1)—each region with 10 isolates—were included.

2009 Monitoring Programme—Discriminating Test System

Sensitivity tests were carried out by EpiLogic, as previously described (Felsenstein 1991; Felsenstein 1994; http://www.frac.info). Spores of *B. graminis* f.sp. *tritici* were randomly trapped in the most important European cereal growing regions and isolates from each region were analysed regarding their sensitivity to metrafenone with discriminatory doses of metrafenone (0.02 and 0.08 mg/l). The formulated product Flexity (300 g metrafenone/l) was used. In total, 2509 isolates of wheat powdery mildew were tested (100 from Ireland, 205 from UK, 130 from France, 30 from Sweden, 60 from Denmark, 1924 from Germany and 60 from Poland). Isolates, which were not fully inhibited by these concentrations, were retested with a range of concentrations (0.00, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24 mg/l) to determine their ED_{50} values. Two strains isolated in the 1970s, possessing a wild-type sensitivity towards all modern active compounds, served as sensitive reference.

Glasshouse Trials

Ten-day-old wheat plants (variety "Riband", 18 seeds per pot with 8 cm diameter) were sprayed in a spray chamber with 1/9, 1/3 and full registered field rate (17, 50 and 150 g a.i./ha) of Flexity (300 g metrafenone/l). The spray volume in this spray chamber corresponded to 400 l water/ha, which was equivalent to concentrations of 42, 125, 375 mg a.i./l. The plants were treated in a preventive and curative fashion, respectively, for several days. Plants were inoculated with three sensitive reference isolates, a moderately adapted and a resistant isolate. After inoculation, plants were incubated 7 days at 18°C and disease was rated in % diseased leaf area on the first two leaves. Three replications (pots) per concentration and isolate were carried out.

Spores from lesions on plants treated with lower (1/9 and 1/3) rates were used as inoculum on untreated leaves in order to analyse the vitality of such spores. Leaves were incubated and evaluated as described above.

Results

Baseline Sensitivity

ED_{50} values of the 140 European isolates collected before the launch of metrafenone ranged from 0.0031 to 0.0124 mg/l with a mean of 0.0053 mg/l. The mean ED_{50} values of the 10 isolates of each region were very similar, ranging from 0.0044 to 0.0061 mg/l.

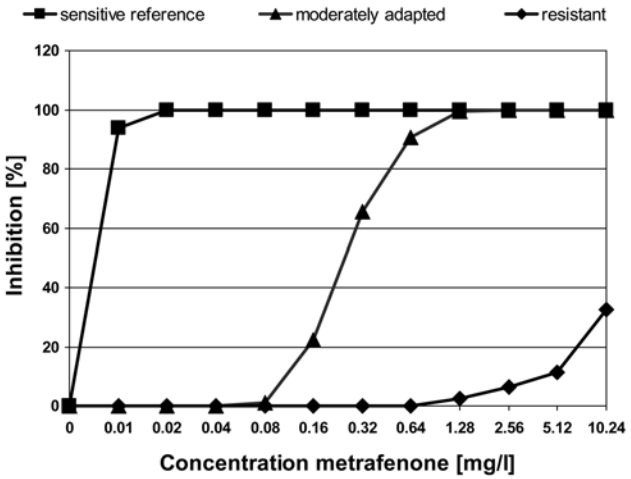

Fig. 1 Inhibition of sensitive reference isolates (mean of 8 measurements: 2 isolates each with 4 independent experiments), moderately adapted isolates (mean of 84 isolates) and resistant isolates (mean of 8 isolates) at various concentrations of metrafenone

2009 Monitoring Programme

Of the 2509 European isolates tested in 2009, the vast majority of isolates (96.3%) were sensitive and completely inhibited at 0.02 mg/l. Ninety-two were not completely inhibited at 0.02 mg/l and also developed powdery mildew symptoms at 0.08 mg/l. These isolates were retested together with 2 sensitive reference isolates with a range of different concentrations. Eighty-four isolates (3.4%) were moderately adapted with ED_{50} values ranging from 0.10 to 0.51 mg/l (mean value: 0.29) representing a mean resistance factor (RF), compared to the baseline values described above, of RF = 55. Eight isolates (0.3%) were classified as resistant with ED_{50} values > 10.24 mg/l (Fig. 1).

Glasshouse Trials

Sensitive reference isolates were completely inhibited at 1/3 of the registered dose rate at any application time point (1 and 4 days preventive as well as 2 and 3 days curative treatment scheme) and also moderately adapted strains were controlled well at full rate in any trial lay-out. A strain classified as resistant showed a decreased response to increasing fungicide concentrations and was not completely controlled even at high dose rates (Fig. 2).

Spores from the 3-day curative trial derived from lesions of untreated leaves and from leaves treated with 1/9 rate (for sensitive isolates) or 1/3 rate (for moderately adapted isolates and resistant isolate) were used as inoculum to investigate the viability of spores from treated leaves. The results indicate that the spores from treated leaves infected with the sensitive and moderately adapted isolates cannot infect untreated leaves, while spores from treated leaves infected with the resistant isolate are able to infect untreated leaves (Table 1).

Discussion

Sensitivity of wheat (*B. graminis* f.sp. *tritici*) and barley powdery mildew (*B. graminis* f.sp. *hordei*) to metrafenone has been monitored after market launch on a yearly basis with *in vivo* tests. Sensitivities (ED_{50} values) have been compared with the baselines established with populations of both *forma specialis* (*tritici* and *hordei*) which were never exposed to metrafenone ("wild-type sensitivity").

No changes in the sensitivity towards metrafenone compared to the baseline sensitivity have been found in extensive studies for barley powdery mildew (*B. graminis* f.sp. *hordei*) up to now (data not shown), indicating full wild-type sensitivity and no adaptation of this *forma speciales* to metrafenone.

The European survey for wheat powdery mildew (*B. graminis* f.sp. *tritici*) in 2009 on the sensitivity of the current wheat powdery mildew population showed that the majority of the population is still highly sensitive to the fungicide metrafenone and on the same level as sensitive strains which were isolated before the introduction of metrafenone.

Some isolates with increased ED_{50} values were identified, which were classified as moderately adapted. Such strains were still controlled with registered field rates in glasshouse trials under various preventive and curative application conditions. Even if some typical powdery mildew lesions occurred in curative treatments with 1/3 of the registered field rate, such lesions did not produce viable spores which would have been able to infect untreated wheat leaves. From these findings it can be assumed that moderately adapted isolates will be controlled in the field with registered rates and that distribution of such moderately adapted isolates is limited, because spore viability is also still affected by metrafenone treatments.

Few isolates with higher resistance factors were identified at a very low frequency (0.3%). Such isolates were not completely inhibited at rates corresponding to the registered dose. The fact that isolates with a high resistance factor are rarely observed indicates that those might possess fitness penalties under natural field conditions. Studies on the competitiveness of such strains with sensitive and moderately adapted strains are currently under way and there are initial indications that the frequency of resistant spores in mixed populations decreases after repeated cultivation on untreated leaves, *i.e.* without selection pressure by metrafenone (data not shown).

Survival of isolates with reduced sensitivity towards a compound in the field is a result of the selection pressure of the fungicide on the one hand and fitness penalties which

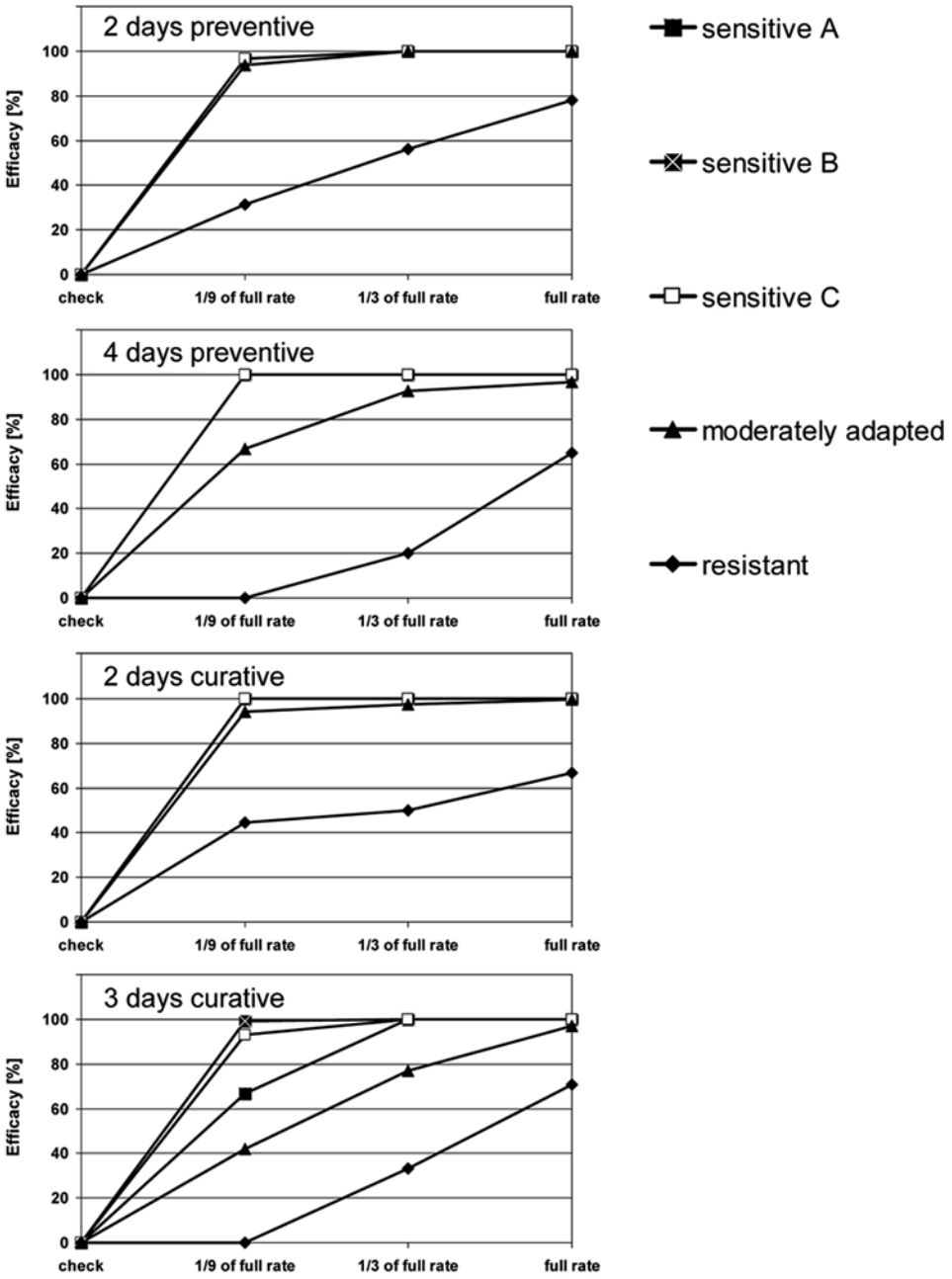

Fig. 2 Preventive and curative activity of metrafenone on isolates with different sensitivities to metrafenone (Three sensitive isolates (labeled with squares), 1 moderately adapted isolate (triangle) and 1 resistant isolate (rhombus) were included)

Table 1 Capability of infection of spores from different isolates taken from metrafenone-treated leaves

Origin of spores	Sensitive isolate	Moderately adapted isolate	Resistant isolate
Spores from untreated leaves	+	+	+
Spores from treated leaves*	−	−	+

Spores of sensitive isolates from leaves treated with 1/9 of the registered field rate were not able to infect untreated leaves. Spores of moderately adapted isolates from leaves treated with 1/3 rate were not able to infect untreated leaves. Spores of a resistant isolate from leaves treated with 1/3 rate were able to infect untreated leaves. Spores of all isolates from untreated leaves were able to infect untreated leaves (+ = spores infected untreated leaves, − = spores did not infect untreated leaves)

*Spores were taken from 1/9 reg. rate for sensitive isolates and 1/3 reg. rate for moderately and resistant isolate

might correlate with the expression of the resistance mechanisms on the other. In order to reduce the selection pressure, effective resistance management programmes are recommended, which should include the use of mixtures with effective fungicides with a different mode of action (*e.g.* morpholine compounds) and/or alternation with other compounds active

against wheat powdery mildew. Since population size of pathogens is lower at disease onset than when already established in the field, selection pressure is less when using preventive applications rather than curative or eradicative spray schemes. Therefore, metrafenone-containing fungicides should be applied in a preventive manner following the recommendations on the product label. Applications should be ensured to be well-timed at the appropriate dose.

For the future, extensive sensitivity monitoring is indicated in order to observe the development of isolates which are outside the baseline sensitivity within the European wheat powdery mildew population.

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