Short Communication

Occurrence of Hyd R3 fenhexamid resistance among Botrytis isolates in Northern German soft fruit production

Vorkommen der Hyd R3 Fenhexamid-Resistenz bei Botrytis im norddeutschen Beerenobst-Anbau

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Abstract
Strains of Botrytis cinerea with a high degree of resistance against the hydroxyanilide fungicide fenhexamid (resistance factor > 2000) were found in strawberry and raspberry fields of the two important Northern German berry fruit production areas around Vechta and south of the Lower Elbe river. The frequency of these Hyd R3 isolates in individual fields was variable, ranging from 0 to 100%. Affected raspberry orchards generally showed a higher proportion of Hyd R3 strains than Hyd R3-affected strawberry fields. It is suggested that repeated applications of fenhexamid over several successive years may have caused an enrichment of Hyd R3 strains in raspberries. Practical implications of these findings for regional soft fruit producers are discussed.

Key words: Botrytis cinerea, fungicide resistance, grey mould, hydroxyanilide, raspberry, strawberry

Zusammenfassung

Stichwörter: Botrytis cinerea, Erdbeere, Fungizid-Resistenz, Grauschimmel, Himbeere, Hydroxyanilid

1 Introduction

Under the mild and humid climatic conditions of Northern Germany, grey mould caused by Botrytis cinerea Pers.:Fr. is one of the most serious fungal diseases of soft fruits such as strawberries and raspberries (Weber and Entrow 2007). In integrated crop production systems, repeated fungicide applications at blossom time are therefore essential for securing a reliable harvest of fruits with an acceptable shelf-life. All fungicides currently registered in Germany for the control of Botrytis and other fungal pathogens of soft fruits have specific modes of action, anilinopyrimidines and, to a lesser extent, strobilurins being in heavy use. In view of the high risk of resistance development in Botrytis against these and other single-site fungicides (Leroux et al. 2002; Myresiotis et al. 2007; Ishii et al. 2009), spray programmes should include compounds with differing modes of action. Fenhexamid, marketed worldwide by Bayer CropScience as Teldor® and other products, is the only member of the hydroxyanilides in current use and has no known cross-resistance with any other fungicide class. It is therefore recommended by most soft fruit consultants as an integral part of the spray sequence against Botrytis.

Resistance against fenhexamid has been reported from vineyards in France where three groups of hydroxyanilide resistance (Hyd R) have been distinguished (Leroux et al. 2002). Hyd R1 and R2 strains showed only a moderate decline in sensitivity to fenhexamid in the laboratory, due at least in part to a metabolism of this compound by vegetative hyphae in rich laboratory media. In contrast, Hyd R3 isolates were highly resistant due to a specific mutation in the erg27 gene encoding the target enzyme for fenhexamid, 3-keto reductase (Fillinger et al. 2008). In the commonly used mycelial growth assays, effective fenhexamid concentrations causing a 50% inhibition (EC50) of Botrytis were 0.1 ppm for baseline strains, up to 3–5 ppm for Hyd R1 and R2 strains, and > 200 ppm for Hyd R3 strains (Leroux et al. 2002; Weber 2010). In contrast, in germ-tube elongation tests only Hyd R3 strains showed a clear-cut resistance response (EC50 > 200 ppm) whereas there were no differences between baseline and Hyd R1/R2 strains after 14 h incubation (both around 0.1 ppm; Weber 2010). Since the elongating germ-tube is the infectious principle and fenhexamid is applied as a protective fungicide, Hyd R3 but not Hyd R1 or Hyd R2 strains are believed to compromise the efficacy of Teldor® in the field (Leroux 2007).

Until recently, no highly fenhexamid-resistant (Hyd R3) strains of B. cinerea had been described in Europe on crops other than grapevine. The discovery of such strains in Northern German strawberry and raspberry plantations (Weber 2010) is therefore a cause for concern. A regional survey was conducted in order to assess the current distribution and abundance of Hyd R3 strains in Northern Germany. These results are reported here, and implications for the further usage of fenhexamid against Botrytis are discussed.

2 Materials and methods

Samples of overwintered plant material showing Botrytis infections were collected in March and April 2009, and fruits with grey mould in June to August 2009. Altogether, 25 strawberry fields and 10 raspberry plantations were sampled, mostly from the two main production areas around Vechta and in the Lower Elbe region separated by a distance of approx. 120 km (see Fig. 1). From each field, at least five viable Botrytis samples were examined.

For an unambiguous characterisation of Hyd R3 resistance, the germ-tube elongation test devised by Weber (2010) was...
used. In outline, after 1–3 d incubation of infected plant samples in a damp chamber at room temperature, macroconidia were harvested directly from sporulating lesions as suspensions in sterile dist. water using a micropipette. Individual drops (20 μl) of the spore suspension were placed on the surface of 1% malt agar plates containing 0, 1 or 50 ppm fenhexamid, and on water agar plates containing 0 or 10 ppm fenhexamid. Conidial germ-tube elongation was assessed by light microscopy after 14 h incubation at 20°C. Only spore samples producing long (> 100 μm), unbranched germ-tubes on all fenhexamid concentrations in both agar media were considered to belong to the Hyd R3 group. In contrast, a stalled germ-tube (< 50 μm) was produced after 14 h by conidia of baseline-sensitive as well as Hyd R1/R2 strains on both agar media augmented with fenhexamid. Germ-tubes of Hyd R1/R2 but not baseline isolates slowly resumed growth after 24–48 h on malt agar with 1 ppm fenhexamid.

Single-spore reference isolates of representative strains of each sensitivity category have been made and are available upon request.

3 Results and discussion

Altogether 270 samples of sporulating Botrytis lesions from 35 fields were examined in a monitoring of the regional distribution of fenhexamid resistance. All but five spore suspensions produced a uniform response and could therefore be unequivocally assigned to the three categories baseline sensitivity (41.5%), reduced sensitivity (Hyd R1/R2; 40.0%) and resistance (Hyd R3; 18.5%). Both the Lower Elbe region and the area around Vechta were affected by Hyd R3 strains of Botrytis which were highly unevenly distributed among the fields sampled, plots with a high proportion of Hyd R3 often being in close proximity to others without such strains (Fig. 1). This observation may reflect the high genetic variability of Botrytis which consists of a site-specific diversity of strains and cryptic species, as reported from other crops (ALBERTINI et al. 2002; KRETSCHMER and HAHN 2008). The quantitative data presented in the current report are the first of their kind published for soft fruits. The very high proportion of Hyd R3 (> 50% in several fields) contrasts with publications from other crops indicating a lower abundance of resistant strains (MA and MICHAILIDES 2005; ESTERIO et al. 2007; MYRESIOTIS et al. 2007). Unfortunately, many of these reported cases of resistance cannot be assigned unequivocally to Hyd R3 because assays of mycelial growth rather than germ-tube elongation were used.

Whilst Hyd R3 strains comprised an average of 64.3% of all isolates in four Hyd R3-positive raspberry orchards, 14 affected strawberry fields yielded only 25.6% Hyd R3 strains on average. This apparent enrichment of Hyd R3 strains in raspberry fields is not surprising because such plots are managed for 10 yr or longer under Northern German cultivation conditions, whereas strawberries are ploughed under after the second or third cropping season. Further, up to four fenhexamid applications were initially recommended for the control of Botrytis in raspberries by the manufacturer (ROSSLENBROICH 1999), and all four heavily affected raspberry plantations had a history of repeated fenhexamid use. This invites the working hypothesis that sporadic contaminations of new fields with Hyd R3 strains (see Table 1) may have been subjected to a site-specific enrichment by selection pressure caused by repeated fenhexamid applications.

Fig. 1: Distribution and frequency of Botrytis strains with Hyd R3 resistance in Northern German raspberry (squares) and strawberry fields (circles). The proportion of Hyd R3 (black segments) relative to the total population is indicated. For each field shown here, at least five samples were analysed. Fields listed in Table 1 are indicated by numbers.