Multiple Fungicide Resistance in *Botrytis*: A Growing Problem in German Soft-Fruit Production

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1. Introduction

In the mild and humid climate of northwestern Europe, fungal pre-harvest rots are a major factor limiting the production of soft-fruits such as strawberries and raspberries. By far the most important fungal disease is grey mould caused by *Botrytis cinerea* Pers.:Fr., now known to be an aggregate of at least two distinct species (Fournier *et al.*, 2005; Giraud *et al.*, 1999). Primary infections are initiated at flowering, resulting in a quiescent colonisation of floral organs (Bristow *et al.*, 1986; Puhl & Treutter, 2008). Upon ripening of the infected fruit, a brown rot becomes apparent (Fig. 1A) which rapidly engulfs the entire fruit, culminating in the production of conidiophores (Fig. 1B). If mild and humid conditions prevail at harvest time, conidia released from fruits with primary infections may infect healthy fruits, causing a catastrophic grey mould epidemic (Fig. 1C). Repeated fungicide applications during flowering are therefore essential to control *B. cinerea* in soft-fruit production.

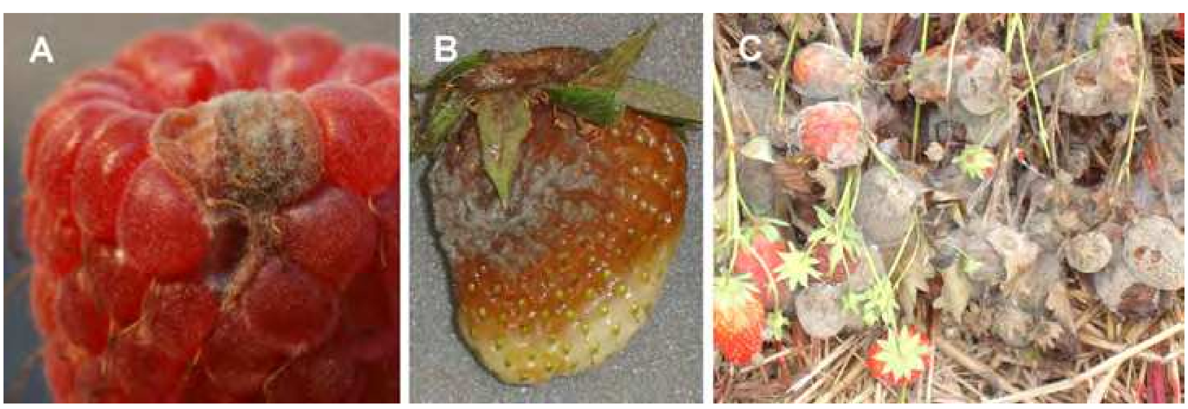

Fig. 1. Stages of *Botrytis cinerea* infections in soft-fruits. (A) Escape of a limited infection from quiescence in a ripening raspberry fruit. (B) Development of a spreading brown rot with the first crop of conidia on an infected strawberry fruit. (C) Occurrence of severe secondary infections during mild and humid weather in a strawberry field in early July 2007.

Northern Germany is a major soft-fruit producing region comprising some 4,000 ha strawberries, 1,500 ha blueberries, 100 ha raspberries and smaller areas dedicated to gooseberry, redcurrant and blackberry production. During the past five years, severe losses to grey mould were recorded by strawberry and raspberry growers. Damage was caused by pre-harvest rot as well as a reduced shelf-life of marketed produce. Although the weather conditions during harvest were often conducive to secondary infections by *B. cinerea*, several fruit farmers also raised concerns about a reduced efficacy of fungicides.

The past four decades have witnessed considerable changes in regional fungicide spray schedules against *Botrytis*. From about 1965 onwards, the sulphamide compounds dichlofluanid and tolylfluanid were widely used as contact (multi-site) fungicides. Although there are literature reports of the development of partial resistance of *B. cinerea* to sulphamides (Malathrakis, 1989; Pollastro *et al.*, 1996; Rewal *et al.*, 1991), no reductions of field efficacy were observed in Northern German soft-fruit production where sulphamides continued to be used as an alternative or complement to a range of specific, single-site fungicides such as benzimidazoles (infrequently used from 1971 to 1976) or dicarboximides (commonly used in 1979-2009). In February 2007, the registration of the last available multi-site fungicide, tolylfluanid, was abruptly withdrawn due to environmental safety concerns. Since then, control of *B. cinerea* has been based solely on compounds with specific (single-site) modes of action, current representatives being fenhexamid (registered since 1999), QoI fungicides (since 2001), boscalid (since 2009), anilinopyrimidines (since 1999) and fludioxonil (since 1999). At present, three widely used botryticides are Switch® (active ingredients cyprodinil + fludioxonil; Syngenta), Signum® (a.i. pyraclostrobin + boscalid; BASF), and Teldor® (a.i. fenhexamid; Bayer CropScience).

Botrytis cinerea is a high-risk pathogen for fungicide resistance development (Brent & Hollomon, 2007; Leroux, 2007), and Northern Germany is a potential 'hot spot' area according to the criteria of Brent & Hollomon (2007). We were concerned about the total reliance on single-site fungicides for grey mould control since 2007, and the lack of any information regarding the current resistance status of regional *Botrytis* populations. In order to address the latter problem, we carried out the surveys and experiments reported here and in previous publications (Weber, 2010b, 2011; Weber & Entrop, 2011; Weber & Hahn, 2011).

2. Fungicide resistance in *B. cinerea*

Botrytis cinerea has earned its reputation as a high-risk pathogen mainly because of its capacity to develop specific resistance to single-site fungicides based on target gene mutations. Specific resistance may emerge within a few years of release of a new fungicide group onto the market, and is usually associated with high resistance factors in laboratory tests. Specific resistance has been described e.g. to benzimidazoles such as benomyl, thiophanate-methyl and carbendazim (Yarden & Katan, 1993; Yourman & Jeffers, 1999), dicarboximides such as iprodione and vinclozolin (Northover & Matteoni, 1986; Yourman & Jeffers, 1999), QoI fungicides (strobilurins; Bardas *et al.*, 2010; Ishii *et al.*, 2009), anilinopyrimidines such as cyprodinil and pyrimethanil (Chapeland *et al.*, 1999; Myresiotis *et al.*, 2007), carboxamides/SDHIs (boscalid; Leroux *et al.*, 2010), and the hydroxyanilide compound fenhexamid (Fillinger *et al.*, 2008; Ziogas *et al.*, 2003). To the best of our knowledge, no reports of specific resistance to the phenylpyrrole compound fludioxonil have been published as yet.

In recent years there have been reports of a non-specific multiple drug resistance (MDR) mechanism based on the activity of ATP binding cassette (ABC) or major facilitator super-family (MFS) transport proteins in *B. cinerea* (Kretschmer *et al.*, 2009; Vermeulen *et al.*, 2001).

MDR phenotypes are usually associated with low or moderate resistance factors, the highest (up to 25-fold relative to baseline sensitivity) being associated with anilinopyrimidines and fludioxonil (Kretschmer *et al.*, 2009).

There is ample evidence that target mutations strongly reduce or even abolish the activity of a fungicide in the field (e.g. Ishii *et al.*, 2009; Myresiotis *et al.*, 2008; Yourman & Jeffers, 1999), and more tentative evidence that this may be the case for MDR phenotypes (Petit *et al.*, 2010; Weber, 2011). Further resistance mechanisms in *B. cinerea* are the metabolisation of fenhexamid (Billard *et al.*, 2011; Leroux *et al.*, 2002) or the role of alternative oxidases in QoI resistance (Wood & Hollomon, 2003), although the impact of either of these mechanisms on the field efficacy of the respective fungicides is far from clear (Leroux, 2007).

2.1 Development of a resistance assay

An assay of conidial germination and germ-tube growth on the surface of agar media was developed by Weber & Hahn (2011). For thiophanate-methyl, iprodione, fludioxonil and fenhexamid, 1% (w/v) malt extract agar was used. In order to exclude fenhexamid-metabolising isolates from being scored as false positives, germ-tube growth was also examined on tap-water agar containing a critical fenhexamid concentration (Weber, 2010a). For a test of the QoI fungicide trifloxystrobin, 1% malt extract agar was augmented with the alternative oxidase inhibitor salicyl hydroxamic acid (100 µg ml^{-1}). For the boscalid and cyprodinil assays, 0.5% yeast extract agar or 0.5% sucrose agar (respectively) were used instead of malt extract agar.

Pure-culture isolates of *B. cinerea* were examined for their response to a wide range of fungicide concentrations in order to determine EC_{50} values under the chosen test conditions (Fig. 2; Table 1). For each fungicide Weber & Hahn (2011) also identified discriminatory concentrations at which highly sensitive or baseline (HS) and less sensitive (LS) isolates could be reliably distinguished from those showing moderate resistance (MR) and high resistance (HR). Examples of dose-response patterns to two fungicide groups are given in Fig. 2.

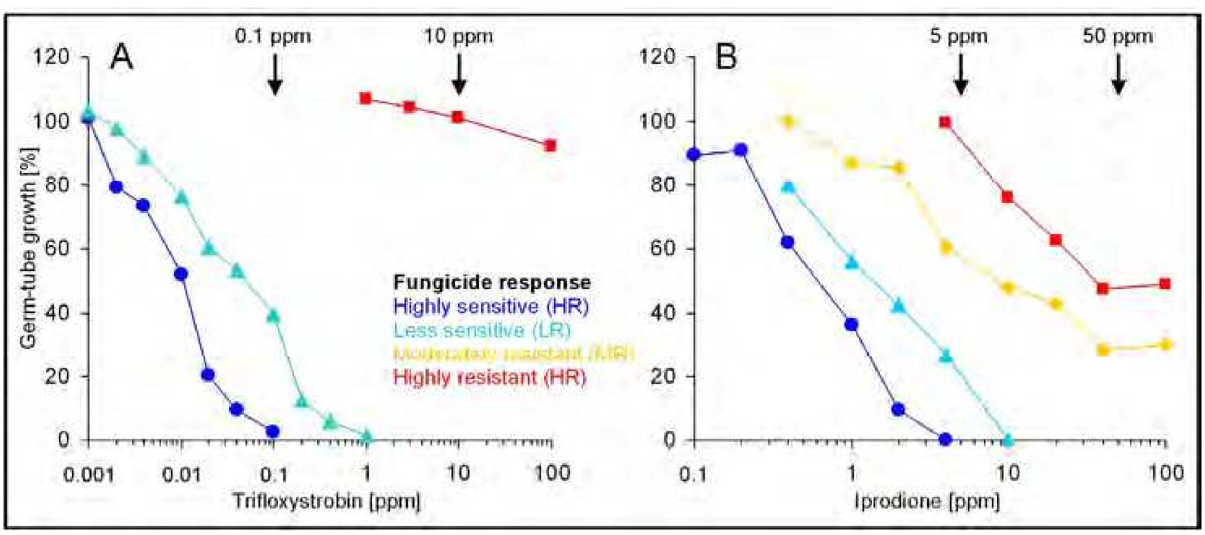

Fig. 2. Responses of selected isolates of *Botrytis cinerea* to trifloxystrobin (A) and iprodione (B), recorded as germ-tube length after 12 h incubation. Discriminatory concentrations for both assays are indicated by arrows. Adapted from Weber & Hahn (2011).

Based on two discriminatory concentrations for each fungicide (Table 1), a routine test was developed as a decision support tool for fruit farmers and their advisors wishing to optimise their fungicide spray sequence for the control of grey mould. This test had to fulfil certain criteria. Firstly, a relatively high throughput of *B. cinerea* isolates had to be possible in order to quantify the abundance of resistant strains in a field. Further, the test had to deliver fast results preferably prior to flowering, precluding lengthy isolation and cultivation periods. Thirdly, the test had to be simple so as to be usable with basic equipment and by non-specialised technical staff associated with general microbiology laboratories.

By obtaining conidial suspensions directly from sporulating overwintered plant samples in early spring or from infected fruits at harvest, plating out 10-15 µl drops of suspension onto each of 20 different agar media, and examining germination and/or germ-tube growth with a light microscope after an overnight incubation (Fig. 3), the method was put to practical use during the 2010 and 2011 growing seasons. A high throughput was achieved by accommodating 20-24 drops of different spore suspensions on each agar plate. Samples were collected by the farmers themselves or by their advisors. Results were generated with this method for individual fields, and were communicated to the fruit farmers within 3-7 days of sampling.

Fungicide	**Medium**	**Fungicide concentration [ppm]**	**EC_{50} [ppm]**			
			HS [1]	**LS**	**MR**	**HR** [2]
Thiophanate-methyl	MEA [1]	0, 1, 100	0.047-0.166	-	4.67-16.1	28.0-436
Iprodione	MEA	0, 5, 50	0.399-0.647	0.976-3.51	9.17-23.7	43.9-96.1
Fenhexamid	MEA	0, 1, 50	0.058-0.229	-	-	>100
Trifloxystrobin	MEA +SHAM	0, 0.1, 10	0.007-0.011	0.019-0.056	-	>100
Boscalid	YEA	0, 1, 50	0.041-0.099	0.116-0.346	-	3.43-54.5
Cyprodinil	SA	0, 1, 25	0.028-0.094	0.267-0.482	0.748-1.44	1.89-22.7
Fludioxonil	MEA	0, 0.1, 10	0.013-0.059	0.104-0.178	0.301-0.647	-

[1] Abbreviations: HR (highly resistant), HS (highly sensitive), LS (less sensitive), MEA (1% malt extract agar), MR (moderately resistant), SA (0.5% sucrose agar), SHAM (100 ppm salicyl hydroxamic acid), YEA (0.5% yeast extract agar)
[2] The HR phenotype of the fenhexamid test on MEA was confirmed by full germ-tube growth on tap-water agar containing 10 ppm fenhexamid.

Table 1. Sensitivity categories and their EC_{50} values in the conidial germination assay of 7 fungicides (summarised from Weber & Hahn, 2011).

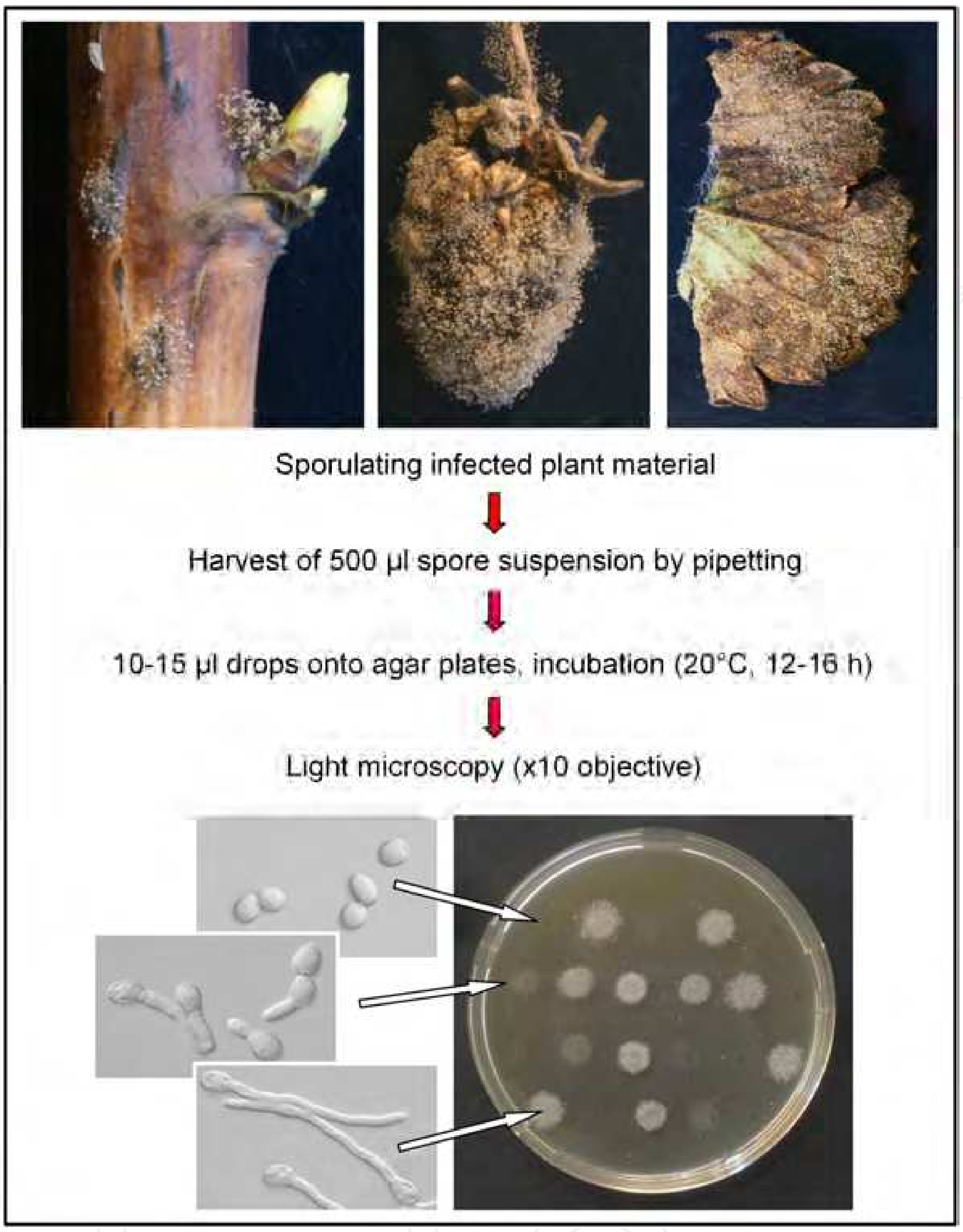

Fig. 3. Outline of the conidial germination assay used for resistance monitoring.

2.2 Reproducibility of the assay method

Overwintered raspberry primocanes infected by *B. cinerea* were collected from selected fields, and incubated in a damp chamber for 2 days. For each cane, conidial suspensions were harvested from three germinated sclerotia separated by a distance of at least 5 cm, and assayed on separate days as described in Fig. 3. The results revealed considerable variations between suspensions obtained from different canes, whereas in most cases the three suspensions collected from any one cane were identical to one another in their fungicide responses (Fig. 4). Further, the observed fungicide resistance phenotypes appeared to be randomly distributed across the field. From these and other results, it was concluded that at least 10 but ideally 15 infected samples should be collected from different parts of the field, and that it was sufficient to analyse one spore suspension from each sample.

Altogether, 93.8% of 1224 conidial suspensions obtained directly from sporulating grey mould lawns on strawberry fruits, raspberry fruits or raspberry canes produced an entirely homogeneous germ-tube growth response to each fungicide, a further 3.4% showing merely minor ambiguities in which less than 10% of conidia differed in their fungicide response from the rest (Weber & Hahn, 2011). The most obvious explanation for such a strong between-sample variation in the near-absence of within-sample variation is that local *B. cinerea* populations may comprise a high genetic diversity with respect to fungicide resistance (Leroch *et al.*, 2011), and that each individual infection is caused by a single germinating conidium.

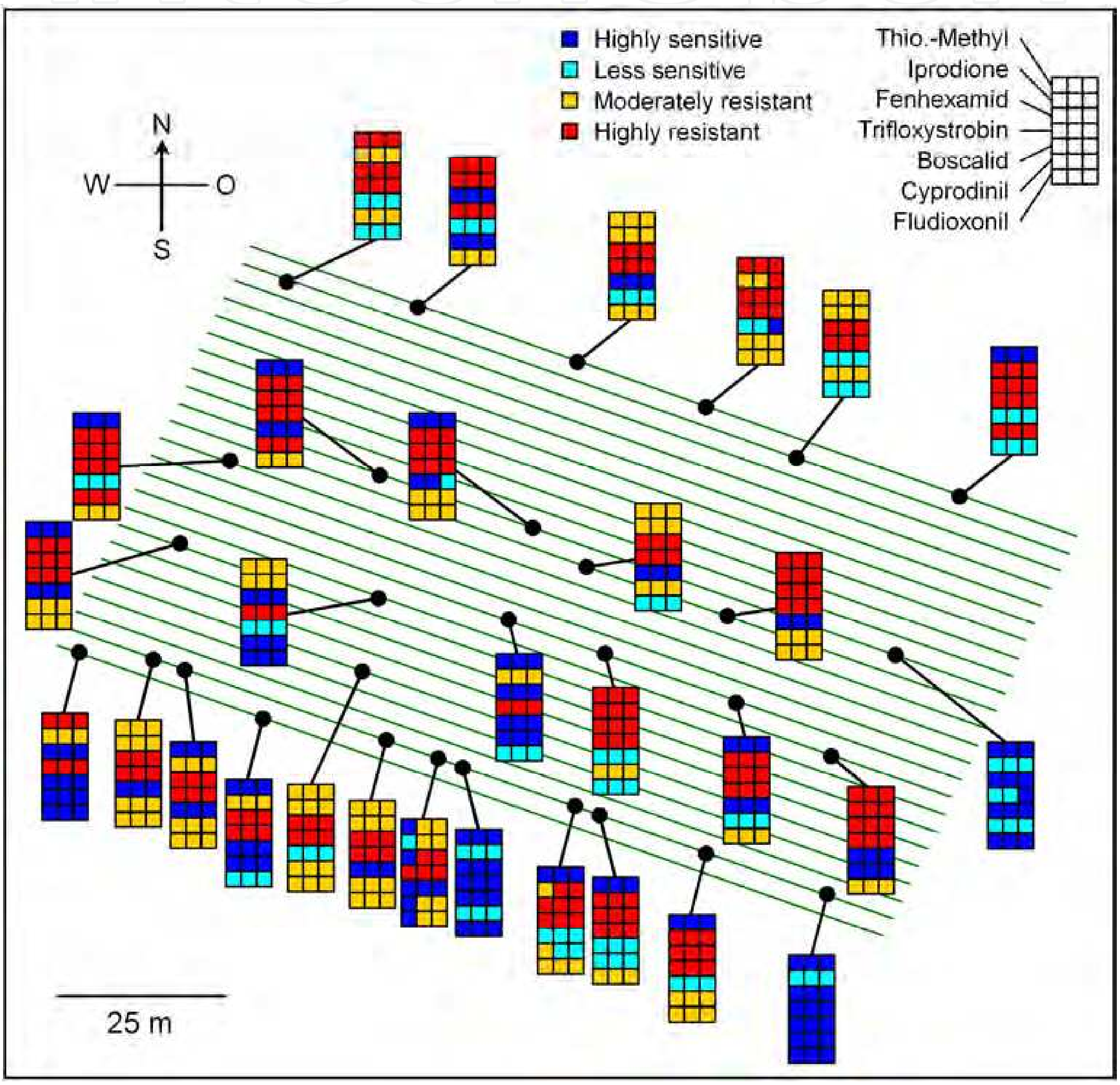

Fig. 4. Reproducibility of the *B. cinerea* resistance assay with 7 fungicides. In April 2010, 30 overwintered infected raspberry canes were collected from a Northern German field at the positions indicated. For each cane, test results of three conidial suspensions obtained from individual germinating sclerotia are shown in columns.

3. Temporal and spatial distribution of fungicide resistance

In a large-scale survey carried out in Northern Germany in 2010, Weber (2011) analysed 353 representative *B. cinerea* conidial suspensions from strawberries, raspberries and other soft-fruits and found that resistance to all 7 fungicides was present at high levels (Table 2). Pure cultures of isolates representing all observed resistance responses to each fungicide were collected. Conidia from MR and/or HR isolates showed a significantly enhanced ability to cause fruit rot in apples pre-treated with the respective fungicides at commercially used concentrations (Weber, 2011), in comparison with sensitive (HS or LS) isolates. In spite of the limitations of the apple inoculation test, these results indicate the possibility that all MR and HR phenotypes identified in the resistance assay might compromise the efficacy of currently registered botryticides in soft-fruit production.

Fungicide	Percent resistant isolates	
	MR	HR
Thiophanate-methyl	18.7%	21.8%
Iprodione	34.8%	29.2%
Fenhexamid	-	45.0%
Trifloxystrobin	-	76.8%
Boscalid	-	21.5%
Cyprodinil	27.2%	14.7%
Fludioxonil	41.1%	-

Table 2. Occurrence of fungicide resistance in Northern German soft-fruit production during 2010 (n = 353 isolates tested). From Weber (2011).

3.1 The dimension of time

In Northern Germany, raspberry fields are being cropped for up to 10 years, rendering them useful objects for long-term studies of resistance development. Results of repeated sampling at two commercial sites are presented in Fig. 5, comparing field A under normal management (3-4 annual fungicide applications during flowering) *versus* field B under an intensive crop protection regime with 6-8 applications (Fig. 5A and Fig. 5B, respectively). Both fields had received 1-2 annual applications of fenhexamid during flowering up to and including the year 2008, but none thereafter. Whilst the share of fenhexamid-resistant isolates collapsed in field A, it remained at a high level in field B. On a regional scale, the percentage of fenhexamid resistance in Northern German soft-fruit production has risen from 18.5% in 2009 (Weber, 2010b) to 45.0% in 2010 (Weber, 2011).

Boscalid resistance is another striking case. This compound was not registered for grey mould control in Northern Germany before the 2009 vegetation period, yet by the end of 2010, *i.e.* its second season of use, there was already a 21.5% share of highly resistant isolates across the region (Weber, 2011). Almost simultaneously, boscalid resistant isolates of *B. cinerea* have been described from other regions and other crops (Bardas *et al.*, 2010; Kim & Xiao, 2010; Leroch *et al.*, 2011).

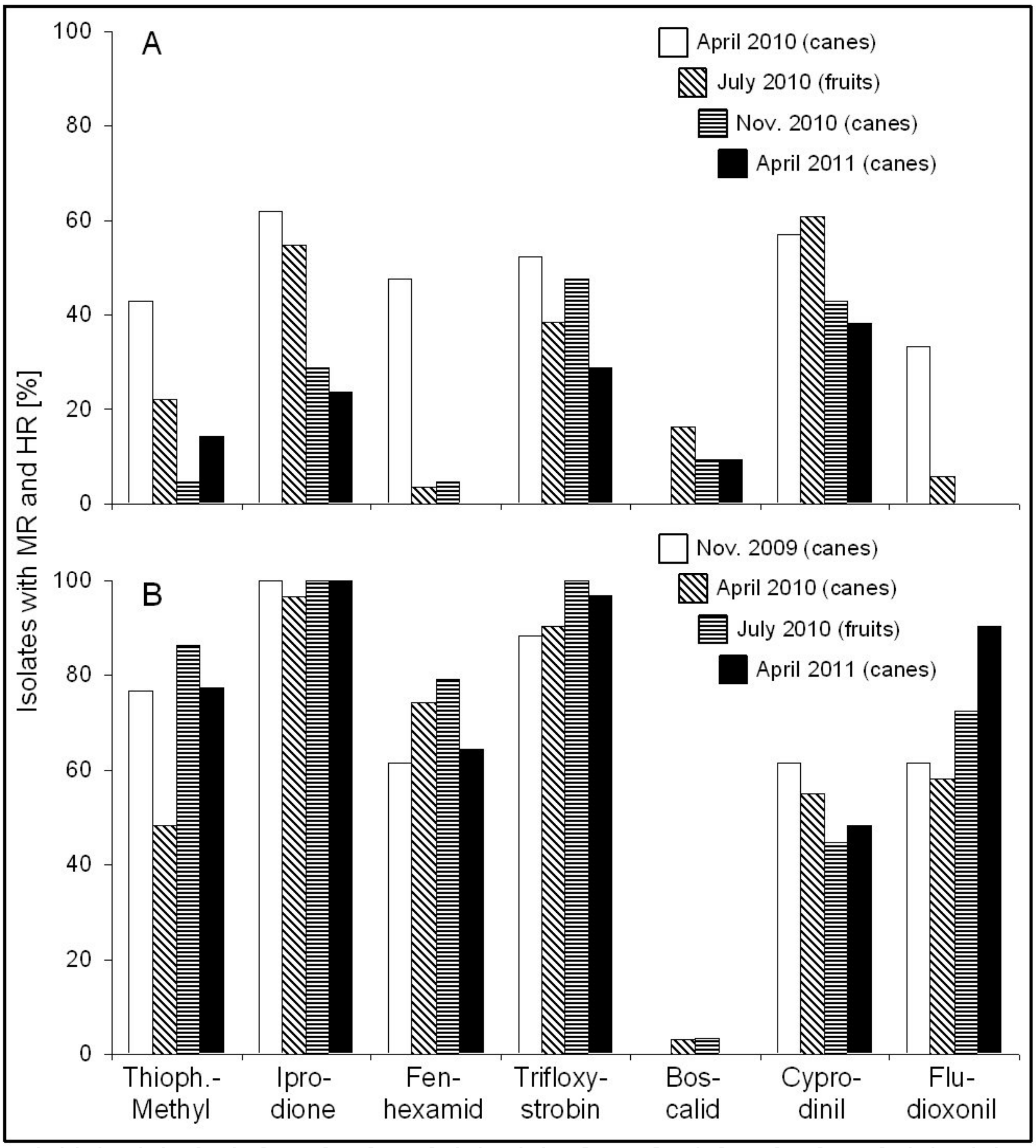

Fig. 5. Percentage of combined moderately and highly fungicide-resistant strains of *B. cinerea* in two Northern German raspberry orchards, one receiving 3-4 annual fungicide sprays at flowering (A), the other 6-8 such treatments (B). In both orchards, fenhexamid was last used in May 2008.

3.2 Selection of resistance by fungicide applications

Tentative correlations between the frequency of fungicide applications and the resulting percentage of resistant strains may be drawn from the results of the 2010 monitoring (Weber, 2011). Unfortunately, only spray schedules for the flowering period immediately preceding sampling were made available by fruit farmers. Nevertheless, for all chemical classes except anilinopyrimidines (cyprodinil, pyrimethanil, mepanipyrim) there was a clear trend towards an elevated share of MR and HR isolates in fields which had received several treatments with the respective fungicides at flowering, as compared to those with the lowest number of applications (Table 3). More detailed analyses incorporating up to 3 years' spray history are being conducted in the course of our 2011 resistance survey.

	Percent MR and HR isolates after 0-6 applications					
Fungicide	**0**	**1**	**1 or 2**	**2**	**3**	**4-6**
Fenhexamid	27.5	46.9	-	59.3	-	-
QoI compounds	-	30.0	-	70.1	91.9	80.8
Boscalid	1.4	20.3	-	25.5	30.4	-
Anilinopyrimidines	-	-	42.7	-	43.1	30.6
Fludioxonil	-	-	14.6	-	55.4	-

Table 3. Percent of fungicide-resistant isolates in comparison with the number of fungicide applications during the flowering period preceding sampling. Evaluation of data collated by Weber (2011).

In order to examine the relationship between fungicide application frequency and resistance development in greater depth, we are performing a series of forced selection experiments (see Brent & Hollomon, 2007). A preliminary trial was carried out in a raspberry orchard during the 2010 season. Variants included (1) a fungicide-free control, (2) four successive sprays of Teldor® as the sole fungicide during flowering, and (3) the farmer's standard spray sequence (Signum® – Switch® – Signum® – Switch®). Resistance tests were conducted with *B. cinerea* isolates obtained from infected fruits at harvest (Fig. 6).

With only 5.7% grey mould-affected fruits at harvest, the farmer's strategy of alternating treatments with two combination products was successful in terms of crop protection relative to the untreated control (18.8% fruit loss to grey mould). However, as indicated in Fig. 6 there was a significant enrichment of *B. cinerea* strains with boscalid resistance (16%) as compared to the other two variants (2.3-4.0%). This result substantiates preliminary observations (Table 3) and literature reports of the unusually rapid spread of boscalid resistance following the release of this fungicide in 2009 (see Section 3.1).

Four fenhexamid applications at flowering provided an acceptable control of grey mould (8.2% diseased fruits at harvest) but resulted in a significant enrichment of HR strains (26%) as compared to the other two variants (1.1-3.5%). A sequence of four successive fenhexamid applications may seem excessive but would have been in line with the manufacturer's original recommendation when this fungicide was first registered for the control of grey mould in raspberries (Rosslenbroich, 1999). Clearly, therefore, a strategy based on repeated treatments with the same single-site fungicide(s) may provide good crop protection during the first year(s) of fungicide use but could turn out to be non-sustainable due to the rapid enrichment of resistant *B. cinerea* strains. In Northern German horticulture there is a veritable history of such boom-and-bust cycles of resistance development within 2-4 years of excessive applications of newly registered fungicides. The best-known examples concern the apple-scab fungus *Venturia inaequalis* which developed resistance to QoI fungicides in 1999 during their third season of intensive use (Palm *et al.*, 2004), and to benzimidazoles in 1974 during their fourth season (Vagt, 1975). Obviously being aware of contemporary reports of benzimidazole resistance in *B. cinerea* from other horticultural situations (Bollen & Scholten, 1971), in a remarkable act of foresight Vagt (1975) cautioned against the continued use of this chemical group for grey mould control in regional soft-fruit production.

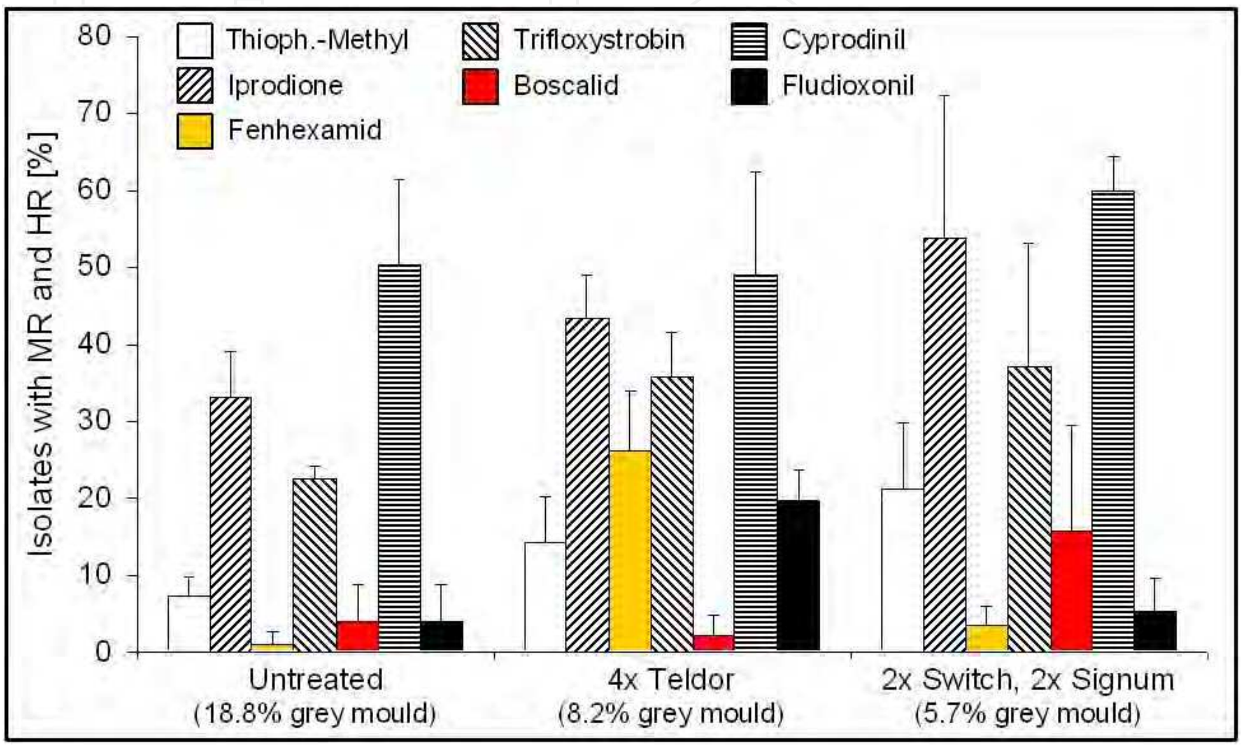

Fig. 6. Forced selection experiment in an 8-year-old raspberry orchard in which plots were left untreated at flowering, or treated with four applications of Teldor® (a.i. fenhexamid) or with an alternating sequence of two Signum® and two Switch® applications. Data are shown as percentage of isolates with MR and HR phenotypes to each of the 7 fungicides tested. Error bars indicate standard deviations of four replicates per treatment. Percentages of fruits with grey mould at harvest are indicated. Altogether 290 isolates were assayed at harvest.

3.3 The issue of multiple fungicide resistance

An important difference between fields A and B shown in Fig. 5 was that strains with multiple resistance were much less common in the former. Thus, only 22.9% of fenhexamid-resistant isolates from field A possessed resistance to more than two of the other four currently used fungicides (QoI, boscalid, cyprodinil and fludioxonil) whereas that share was 49.4% in field B. A situation is therefore conceivable in which strains with resistance to a given fungicide may be enriched or maintained in a field even if that compound is no longer used. Such interactions must be taken into consideration when evaluating the 'fitness' of different fungicide resistance phenotypes.

In general terms, there seems to be a trend in *B. cinerea* towards accumulating resistance, as shown also in the large-scale survey by Weber (2011) who grouped his isolates according to multiples of resistance (0 to 5) to the five currently used fungicide groups, *i.e.* fenhexamid, QoI compounds, boscalid, anilinopyrimidines, and fludioxonil. There was a significant positive correlation between the number of resistances to current fungicides and the proportion of strains harbouring resistance to iprodione or thiophanate-methyl, two compounds no longer in use. Furthermore, all isolates with quintuple resistance (MR or HR) to current fungicides were obtained from fields with a history of unusually heavy fungicide use.

It is of interest to re-interpret the combinations of resistances in individual isolates (Weber, 2011) in terms of their likely impact on the field efficacy of the commercial products Switch®, Signum® and Teldor®. Thus, even when adding up all fully sensitive isolates as well as those with sensitivity to at least one of the two active ingredients in Signum® or Switch®, merely 38% of all isolates obtained in the 2010 monitoring would have been susceptible to all three products, 40.5% to two, 15% to one, and 6.5% to none of them (Table 4). These data should ring alarm bells with fruit farmers, advisory services and regulatory authorities, especially if a trend towards a further increase in resistance development can be recognised in the results of the ongoing 2011 survey.

Sensitivity [1] to	**Percent of isolates [2]**
Teldor + Signum + Switch	37.95%
Signum + Switch	24.65%
Teldor + Signum	3.68%
Teldor + Switch	12.18%
Signum only	12.18%
Switch only	2.27%
Teldor only	0.57%
none	6.52%

[1] Meaning sensitivity to at least one of the active ingredients of the commercial product
[2] Total = 353 isolates analysed

Table 4. Proportions of *Botrytis* isolates from Northern German soft-fruit fields with putative field sensitivity to the commercial fungicides Switch® (a.i. fludioxonil and cyprodinil), Signum® (a.i. pyraclostrobin and boscalid) and Teldor® (a.i. fenhexamid), as extrapolated from the resistance tests of Weber (2011).

3.4 Factors favouring the spread of fungicide resistance in *B. cinerea*

From this chapter as well as previous publications (Weber, 2010b, 2011), it is apparent that *B. cinerea* populations associated with Northern German strawberry and raspberry fields harbour an exceptionally high proportion of MR- and/or HR-type resistance against all currently registered fungicide classes, and that resistance development may be on the increase. We are particularly concerned about the possible spread of strains with multiple fungicide resistance because such a development questions the sustainability of the current crop protection practices.

There are several possible reasons to account for the spread of fungicide-resistant strains of *B. cinerea*. Firstly, since February 2007 grey mould control has been based solely on single-site fungicides, all of which except fludioxonil are known to be susceptible to specific resistance development based on target gene mutations. Secondly, the number of fungicide applications made by fruit farmers during flowering has often exceeded regional recommendations (Weber & Entrop, 2011; see Fig. 5). Autumn-cropping strawberries are of particular concern in this respect because their prolonged flowering periods necessitate more numerous fungicide treatments. Thirdly, the short permissible pre-harvest intervals of 3-7 days for the registered fungicides Switch®, Signum® and Teldor® may have encouraged farmers to spray them against ongoing grey mould epidemics between successive fruit pickings at harvest. Fourthly, restrictions by food retail chains concerning the maximum permissible number of detectable pesticide residues in soft-fruits have forced fruit farmers to abandon resistance management strategies in favour of repeated applications of only one or two of the three main botryticides Switch®, Signum® and Teldor®. As we have seen, there is preliminary experimental evidence that such a strategy may lead to a specific build-up of resistance in selected fields within a single vegetation period (Fig. 6). Fifthly, promises of high degrees of efficacy of these botryticides by agrochemical companies may have encouraged fruit farmers to neglect non-chemical crop protection measures related to sanitation, irrigation, aeration and fertilisation.

4. Recommendations

There are several ways to support the activity of fungicides in grey mould control. Most of these complementary approaches are matters of basic horticultural knowledge (see e.g. Sutton, 1998; Weber & Entrop, 2011). All of the suggestions made below may not be applicable to all fruit farmers, but most farmers should be able to implement some of them.

4.1 Fungicide applications

The number of fungicide applications during flowering should be limited to 3-4 sprays at intervals of approx. 7 days. Additional applications do not generally lead to a higher efficacy of grey mould control under Northern German conditions (Faby, 2009) and are counterproductive in terms of resistance management. All three available botryticides should be used, unless site-specific tests have indicated the presence of resistant strains at frequencies at or above 25% (Forster & Staub, 1996; Petit *et al.*, 2010; Weber, 2010a). Switch®, Signum® and Teldor® should not be applied at harvest, as post-harvest treatments e.g. of cane diseases, or indeed at any time of year other than flowering. In view of the widespread resistance to QoI fungicides in *B. cinerea* and their limited efficacy against grey mould, this fungicide class should not be applied singly during flowering.

4.2 Registration of new fungicides

In general terms, broad-spectrum fungicides such as tolylfluanid, chlorothalonil, folpet or thiram possess a lesser efficacy against *B. cinerea* than single-site fungicides (Gullino *et al.*, 1989; Singh & Milne, 1974; Weber & Entrop, 2011), but they are also less susceptible to resistance development (Leroux, 2007). Future experiments should focus on examining their impact on the development of resistance against the current single-site fungicides when applied as tank mixtures. Detailed experiments under regional conditions are required because previous workers have obtained heterogeneous results (Gullino *et al.*, 1989; Hunter *et al.*, 1987; Northover & Matteoni, 1986). If a suitable broad-spectrum fungicide can be found, this should be registered for grey mould control with some urgency.

Similar trials should also be performed with alternative control agents such as inducers of systemic acquired resistance or biological control organisms. As with broad-spectrum fungicides, efficacy should be evaluated as an effect on the spread of fungicide resistance, rather than (or in addition to) grey mould control *per se*.

4.3 Issues of cultivation

Crop cultivation should aim to maximise yields as well as create conditions which optimise fungicide efficacy. Nitrogen fertilisation of strawberries should be reduced to about 60 kg ha^{-1} *per annum* which is sufficient to ensure a good yield whilst avoiding soft plant tissues susceptible to *B. cinerea* infections. Periods of leaf wetness should be reduced to a minimum by using drip irrigation if possible, or alternatively by using rather than extending natural periods of leaf wetness for overhead irrigation. Strawberry or raspberry crops grown in poly-tunnels or under fleece for early cropping should be aerated regularly in order to reduce periods of leaf wetness. Likewise, the planting distance should be sufficient to ensure a good ventilation of leaves and flowers.

Hygiene measures aimed at reducing inoculum should be given a high priority. Thus, rotting or mouldy fruits should be collected separately and removed from the field especially during the first pickings of the season. Likewise, infected raspberry canes should be pruned and removed from the field before bud burst.

4.4 Outlook

In contrast to pome fruit farming, there is no commercially relevant organic soft-fruit production in Germany, the chief reason being dramatic pre- and post-harvest losses to grey mould. Clearly, non-chemical crop protection measures alone are insufficient to provide acceptable control of *B. cinerea* in Northern Germany. However, as we have discussed in the present chapter, a similar situation may soon hold for the current crop protection strategy based on specific fungicides. Fungicide resistance development in *B. cinerea* should catalyse a change of paradigm towards a truly integrated crop protection concept embracing both chemical and supplementary non-chemical measures. There should be ample rewards for fruit farmers willing to embark on such a concept because of a stable demand for fresh and regionally produced soft-fruits on the market.

5. References

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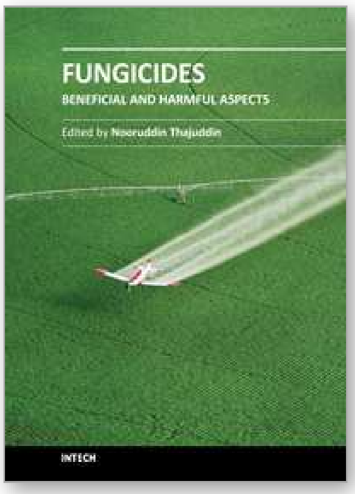

Fungicides - Beneficial and Harmful Aspects

Edited by Dr. Nooruddin Thajuddin

ISBN 978-953-307-451-1

Hard cover, 254 pages

Publisher InTech

Published online 16, December, 2011

Published in print edition December, 2011

Fungicides are a class of pesticides used for killing or inhibiting the growth of fungus. They are extensively used in pharmaceutical industry, agriculture, in protection of seed during storage and in preventing the growth of fungi that produce toxins. Hence, fungicides production is constantly increasing as a result of their great importance to agriculture. Some fungicides affect humans and beneficial microorganisms including insects, birds and fish thus public concern about their effects is increasing day by day. In order to enrich the knowledge on beneficial and adverse effects of fungicides this book encompasses various aspects of the fungicides including fungicide resistance, mode of action, management fungal pathogens and defense mechanisms, ill effects of fungicides interfering the endocrine system, combined application of various fungicides and the need of GRAS (generally recognized as safe) fungicides. This volume will be useful source of information on fungicides for post graduate students, researchers, agriculturists, environmentalists and decision makers.