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Learning from *Botrytis* Monitoring after more than 20 Years of Switch®

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INTRODUCTION

Botrytis cinerea, causing gray mold, a disease difficult to control on a large variety of crops, is a high risk pathogen for fungicide resistance development. Amongst the marketed solutions for *Botrytis* control, Switch® (a mixture of cyprodinil and fludioxonil) displays exceptional stability in terms of performance and remains a market standard despite being used for more than 20 years.

European monitoring results, mostly from Syngenta trials and gathered over the past 6-13 years, enable the assessment of different situations across crops and spray regimes. The molecular identification of multi-drug resistance (MDR) mechanisms (Kretschmer et *al.* 2009; Leroch et *al.* 2013), responsible for shifted sensitivity towards both fludioxonil (FDL) and cyprodinil (CDL) components of the mixture, and of the recent elucidation of specific mechanisms responsible for resistance towards the anilinopyrimidines fungicides to which CDL belongs (unpublished data), enabled us dissecting the resistance situation more precisely. In particular we tested the impact of the different mechanisms either separate or in combination in *in planta* efficacy tests. An overview of selection and efficacy gathered from multiple field trials performed in 2015 enables us to further draw conclusions in terms of robustness, resistance evolution, and application recommendations.

RESULTS AND DISCUSSION

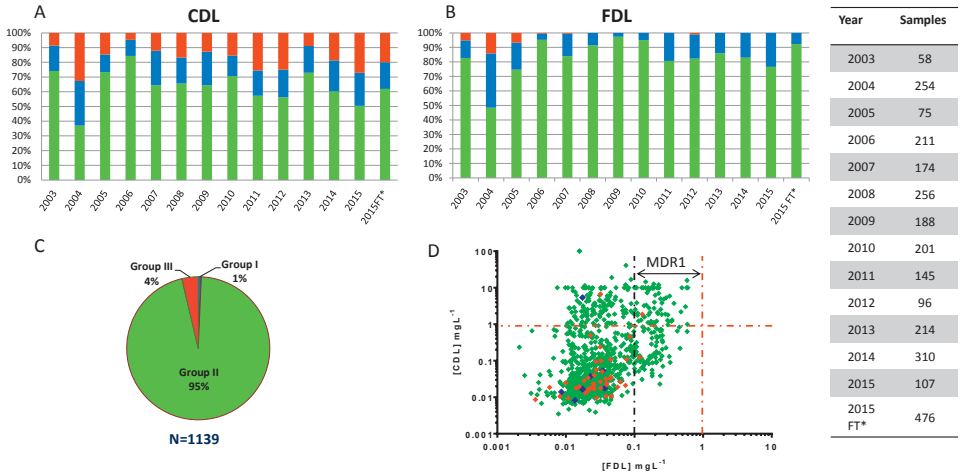
Evolution of cyprodinil and fludioxonil sensitivities in Europe

Resistance evolution in grapes

Botrytis cinerea monitoring performed over the years in Europe suggests overall stability in the frequency of CDL and FDL shifted isolates in grapes (Fig. 1). For both compounds, isolates were classified as slightly shifted when displaying EC_{50} values between 0.1 mgL^{-1} and 1 mgL^{-1} and resistant when displaying EC_{50} >1 mgL^{-1} in gelatine glucose media liquid culture tests.

For CDL (Fig 1A), the frequency of resistant isolates varied between 5% and 30% depending on the year. This frequency mostly reflects the presence of CDL-specific resistance, but also partly the presence of MDR1 which can be found either solo or combined with the presence of

CDL-target resistance mutations in the sample. For FDL (Fig 1B), the frequency of resistant isolates displaying EC_{50} >1 mgL^{-1} is very low since 2006. So far we didn't observe target mutations conferring resistance to FDL in *Botrytis* field isolates, but such mechanism was recently suggested from Chinese samples collected on cucumber and tomatoes (Ren et *al.* 2016). The highly shifted isolates found in 2003-2005 have not been sequenced at the *Bos1* locus, therefore we cannot exclude this mechanism completely, even though such resistance mechanism so far have not been reported in Europe. The grapes' population displays moderately shifted isolates with EC_{50} comprised between 0.1 mgL^{-1} and 1 mgL^{-1}. The shift was shown to be the result of MDR1-type *Mrr1* mutations (Kretschmer et *al.* 2009; Scalliet et *al.*, unpublished data). The frequency of these moderately FDL-shifted isolates is comprised between 3 and 24% for the whole period and stabilized at ~20% since 2007. The population is mostly composed of *B. cinerea sensu stricto* and we observed a very low occurrence of *B. cinerea* group S isolates on grapes (Fig 1C) (Leroch et *al.* 2013).



Year	Samples
2003	58
2004	254
2005	75
2006	211
2007	174
2008	256
2009	188
2010	201
2011	145
2012	96
2013	214
2014	310
2015	107
2015 FT*	476

Figure 1 Relative frequency of sensitive (green, EC_{50} <0.1 mgL^{-1}); intermediate (blue, EC_{50} >0.1 and <1 mgL^{-1}) and resistant (red, EC_{50} >1 mgL^{-1}) isolates of *B. cinerea* from European grapes monitoring regarding cyprodinil (CDL, panel A) and fludioxonil (FDL, panel B) respectively. FT* isolates originate from specific field trials described in section 1.2. The numbers of tested samples per year are listed in the table on the right. Panel C represents molecular partition of the grapes' population based on *Mrr1* PCR assays, group I is *B. pseudocinerea*, group II is *B. cinerea* sensu stricto, group III is *B. cinerea* group S. Panel D displays a cross resistance plot showing the presence of MDR1 isolates (vertical black dotted line) with or without anilinopyrimidine resistance (horizontal dotted line). Note the absence of MDR1h isolates in grapes.

Despite an annual variation that can be observed, since 2005 the overall situation looks stable for both active ingredients, suggesting appropriate application recommendations in grapes. Furthermore, the frequency of isolates carrying both MDR1 and CDL-specific resistance is only ~7% showing that a minor fraction of the population is shifted towards both active components of Switch® (Fig 1D).

Resistance evolution in strawberries

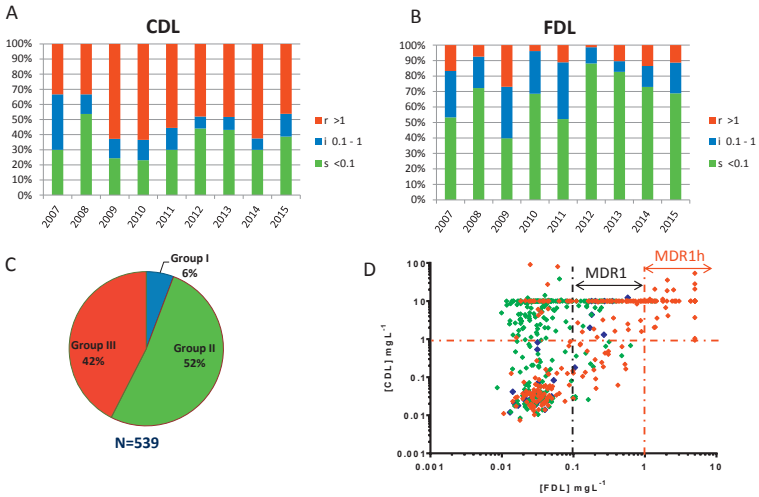
Monitoring was performed in European strawberry fields since 2007 (Fig.2). The frequency of resistant isolates was found to be much higher compared to grapes. For CDL (Fig 2A), the frequency of resistant isolates varied between 32 and 62%, and was usually around 50%, due to a much higher frequency of target-based anilinopyrimidine resistance (unpubl. result). For FDL (Fig 2B), the frequency of MDR1-shifted isolates was also higher compared to grapes, not only moderately shifted (MDR1), but also highly shifted (MDR1h) isolates (EC_{50} >1 mgL^{1}) could be found at high frequency in some years (up to 28% in 2009). The strawberries' population was distinct from that in grapes, and was almost equally devided between *B. cinerea sensu stricto* and *B. cinerea* group S isolates (Fig 1C). MDR1h conferring FDL resistance was exclusively observed in group S isolates. Overall, the occurrence of FDL resistance was above 10% and a high level of variation was found across Europe. Especially samples from Germany displayed a high frequency of FDL shifted isolates, the underlying mechanisms were all found to be caused either by MDR1 or MDR1h mutations as validated by the sequencing of the *Mrr1* gene (Leroch et *al.* 2013; Scalliet et *al.*, unpublished result).

Evidence for selection after multiple applications

During the 2015 season, a dedicated monitoring was performed at multiple locations on grapes field trials. Parallel sampling was performed, on both untreated and Switch®-treated plots (2 to 3 applications). Twelve strains were isolated per condition and assessed for their sensitivity towards CDL and FDL in liquid culture tests.

Overall, the evidence for selection of shifted isolates was low on Switch®-treated plots (Fig 3). Globally, only a slight shift in the median for CDL could be observed after Switch® treatment (0.027 to 0.063 ppm) and a very slight increase in the frequency of FDL-shifted isolates could be seen without a clear effect on the median (0.020 to 0.023 ppm). Furthermore, in 7 of the sites very rare CDL-resistant isolates (up to one per sample) could be found in untreated plots and Switch® treatment did not increase this frequency in 6 out of the 7 trials. There was also no evidence for MDR1 as could be seen from the very narrow range of EC_{50} values for FDL, all below 0.1 mgL^{-1} on both, treated and untreated plots.

Conversely, in 13 of the trial sites, CDL resistance was found on untreated plots at higher frequencies (2 or more out of 12 isolates) and the increased frequency of such genotypes was clear on Switch®-treated plots. The pre-occurrence of MDR1 was suggested by the high frequency of FDL-shifted (MDR1) isolates on untreated plots at 5 locations. The effect of Switch® treatment on the median value for FDL was minor in all but one of these cases. Overall the occurrence of high frequency of CDL resistance on untreated plots seemed to be correlated to the potential appearance or co-selection of MDR1 isolates suggesting anilinopyrimidine resistance precludes the selection of MDR1.



Year	Samples
2007	30
2008	54
2009	78
2010	156
2011	90
2012	152
2013	58
2014	40
2015	106

Figure 2 Relative frequency of sensitive (green, EC_{50} <0.1 mgL^{-1}); intermediate (blue, EC_{50} >0.1 and <1 mgL^{-1}) and resistant (red, EC_{50} >1 mgL^{-1}) isolates of *Botrytis* spp. from European strawberries monitoring regarding cyprodinil (CDL, panel A) and fludioxonil (FDL, panel B) respectively. The numbers of tested samples per year are listed in the table on the right. Panel C represents molecular partition of the strawberries' population based on *Mrr1* PCR assays, group I is *B. pseudocinerea*, group II is *B. cinerea* sensu stricto, group III is *B. cinerea* group S. Panel D displays a cross resistance plot showing the presence of MDR1 isolates (vertical black dotted line) with or without anilinopyrimidine resistance (horizontal dotted line) and the presence of MDR1h isolates (vertical red hatched line) which are exclusively of the group S (red diamonds).

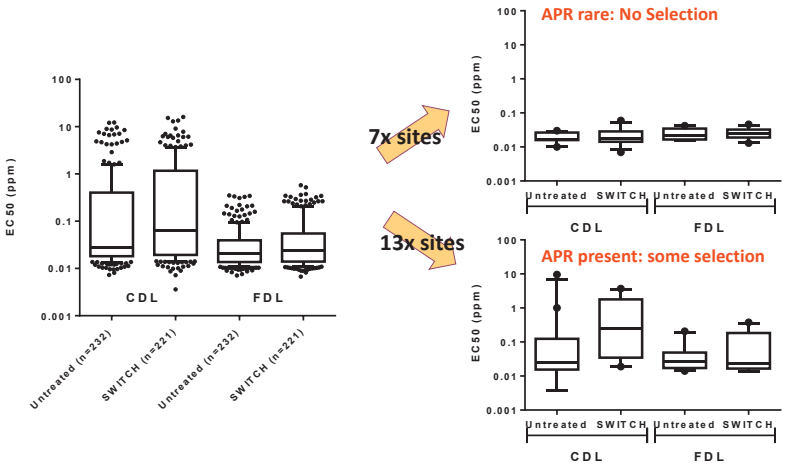


Figure 3 Sensitivity range of samples collected from 20 grapes field trials (2015) (10-90 percentiles box plots) and examples of the two main situations observed. APR: anilinopyrimidine resistance.

Results from *in* planta tests

In planta efficacy tests were performed with strains carrying either CDL target resistance or MDR1 or the combination of both mechanisms. In order to measure their contribution to the efficacy of the product, the two components of Switch® were tested at doses found in the mixture (Fig 4). As could be expected from *in vitro* evaluations, CDL target resistance was completely controlled by the fludioxonil component of Switch® *in planta*. MDR1 showed a moderate effect on both field rates of cyprodinil and fludioxonil components, but the efficacy of the mixture was fully maintained. When CDL target resistance was combined with MDR1, a slight reduction in efficacy could be observed, but the product was still highly effective with >75% activity. These results are nicely showing that CDL brings additional activity in the mixture in the control of MDR1 strains even in the presence of CDL target resistance.

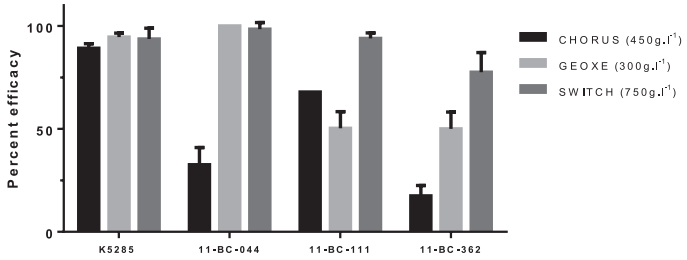


Figure 4 Efficacy results from *in planta* sensitivity test (tomato plants, evaluation 3 DAI). Plants were either treated with Chorus® 50WG (solo formulation of cyprodinil), or Geoxe® 50WG (solo formulation of fludioxonil) which are the respective rates included in Switch® 62.5WG. Genotypes of tested strains are as follow: strain K5285 is wild type, strain 11-BC-044 is CDL resistant, strain 11-BC-111 is MDR1, and strain 11-BC-362 is CDL resistant and MDR1.

Results from European efficacy trials.

The efficacy of Switch® in gray mold control has been controlled in 16 field trials done in Europe in 2015. In these trials the mixture of cyprodinil and fludioxonil has been applied twice and compared to a new recently registered SDHI (Fig. 5). The efficacy results confirm the high practical relevance of Switch® in the control of *Botrytis* in grapes. Under an average pressure of 19% gray mold severity on bunches in the untreated plots, its efficacy level remained similar to the best standard currently registered with a mean of 74% of control compared to 72% for the new compound.

CONCLUSIONS

Switch® displays outstanding robustness because the two active ingredients with different modes of action protect each other from efficacy losses. In particular, the FDL component in the mixture enables the effective control of CDL target mutants. Both CDL and FDL are moderately impacted by MDR1, the combination of the two active ingredients display

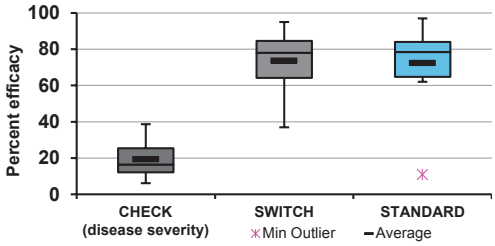


Figure 5 Box plot representation of efficacy results from 16 field trials conducted in Europe in 2015. Y axis represents either percent disease severity on bunches in the untreated plots (check), or percent efficacy either for Switch® or for recently registered SDHI (standard).

additional effects leading to the full control of MDR1 strains and to a good efficacy when MDR1 is combined to the presence of CDL target resistance. Populations in grapes and strawberries are very distinct, in grapes only MDR1 is found and the frequency of multiresistant strains carrying combined MDR1 and CDL resistances is low (around 7%). In strawberries, the presence of group S strains displaying a higher MDR1 phenotype (MDR1h) confers FDL resistance. The frequency of combined CDL target resistance and MDR1h in strawberries is around 10%. To avoid the dominance of strains carrying MDR1(h) phenotypes and to guarantee maintained high efficacy of Switch® it is important to maintain both anilinopyrimidine target resistance and MDR1(h) at reasonably low levels in *Botrytis* field populations. Ideally no more than two Switch® applications per season should be used and in such cases the usage of solo anilinopyrimidines should be avoided in order to prevent the accelerated selection of both CDL-target resistance and MDR1(h) isolates. The same should logically apply to fludioxonil, which should not be used more than twice a season unless mixed with an effective fungicide partner not selecting for MDR1(h).

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