

## Control of early blight by the use of SDHI fungicides

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### SUMMARY

Regarding the increasing amount of SDH-mutated *Alternaria solani* isolates, it's necessary to get a deeper knowledge about the influence of these mutations on fungicide efficacy of different SDHIs (Succinate dehydrogenase inhibitors). Therefore all three stages of sensitivity-testing were performed: *in vitro* trials (calculation of the EC50 values), *in vivo* trials (greenhouse trials with boscalid) and last but not least field trials (fungicide efficacy of different SDHIs under field conditions with two potato varieties). For the field trials it was also essential to get a targeted infection with mutated *A. solani* isolates to observe the real interaction between the fungicide and the mutated field isolate on the field. Therefore the kernel infection with two mutated isolates (both H134R) and one SDH-wildtype isolate was used. All in all, a decreasing fungicide efficacy has been observed in all three stages of the testing. Interestingly different fungicide performances were observed regarding the kernel infections with the two mutated isolates (both H134R). In the field trial it was also reassured that the variety still plays an important role in integrated pest management.

### KEYWORDS

*Alternaria solani*, early blight, SDHI fungicides, control of early blight, SDH-mutation, fungicide resistance, kernel infection, fungicide sensitivity testing

### INTRODUCTION

In recent years the increasing relevance of *Alternaria solani*, the pathogen which causes early blight on potato, leads to several discussions about the best way to control this disease. The most effective way of controlling this pathogen is the use of fungicides. There are two main fungicide groups, the QoIs and the SDHIs. Beside the shift in fungicide sensitivity of *A. solani* against the QoIs (Leiminger et al., 2013; Pasche et al., 2004), there is also an increasing resistance development against the SDHI Boscalid reported in the last few years (Gudmestad et al., 2013, Bauske et al., 2017). In case of the SDHIs, the decreasing fungicide efficacy can be traced back to some mutations in the subunits of the succinate dehydrogenase of *A. solani*, named B-H278R/Y, C-H134R, D-D123E and D-H133R. The upcoming question is now, if and in which dimension these mutations have an influence on the efficacy of SDHI-fungicide treatments. To answer this question *in vivo* and field trials were performed with different SDH-mutated and wildtype isolates. *In vitro* trials were already performed for these isolates.

## MATERIALS AND METHODS

### *In vivo trial*

For the greenhouse trial the cultivar Kuras was used due to its high susceptibility against *A. solani*. The plants were cut to a three-leaf stadium and then treated with different amounts of boscalid (0; 0,1; 1; 10; 100µg boscalid/ml) one day before inoculation with the fungus. In this trial three plants per treatment were used. Three neither treated nor inoculated control plants were also integrated. After the inoculation with the spore solutions of the different *A. solani* isolates, the plants had to incubate in the mist chamber for 48 h at 100% relative humidity and 20°C. After these 48 h a relative humidity of 70% was pursued until the end of the experiment. The experiment was performed with three SDH-wildtype isolates, two B-H278R mutants, two C-H134R mutants and two isolates with a not yet described mutation in the subunit C. The affection with *A. solani* was assessed after two and seven days after inoculation (dai). For the visual assessment the rating schedule from Granovsky and Peterson (1954) was used. The trial was repeated three times for most of the isolates. Two isolates were only repeated twice, due to growing problems.

### *Field trial*

#### *General information*

For the field trial in Freising - Weihestephan, Germany, the cultivars Maxilla from the late maturity group, known to be susceptible, and Lady Amarilla from the early maturity group, known to be very susceptible against early blight were used. The plot size was 4m x 4,5m. The trial consists of four different treatments with commercial products, which differ in their active ingredient and an untreated control. The trial included four repetitions per treatment. Furthermore, a kernel infection with three different *A. solani* isolates were done on the one hand to ensure an infection with early blight and on the other hand to have separated SDH- wildtype and -mutant *A. solani* populations in the field. So, for the kernel infection one SDH-wildtype isolate and two SDH-mutants were used.

#### *Kernel infection*

The used isolates were cultivated on SN-agar for two weeks under near UV-light (12h/12h). To generate the kernel inoculum, 150g of barley kernels were put in an autoclavable bag with 60 ml distilled water and closed with a buckler and rubber band. The bags were autoclaved twice. Half of an overgrown, about 2 weeks old SNA-plate was used to knead the kernels with the grown fungus, to ensure adherence of the conidia on the grains in the autoclaved bag. The infected kernels need to be incubated for four weeks under near UV-light (12h/12h), to support fungal growth. 5 g of the finished kernels were spread equally per m<sup>2</sup> between the potato rows. The trial was split into three parts, one for the wildtype inoculation, one for the mutant 1 and another one for mutant 2, to minimize an intermixture of the different isolates by wind or rain splash.

#### *Disease assessment*

The observation of the disease progress started with emergence until death of the potato plants. In each of the four replications, 10 plants per plot were rated for disease progress of early blight. To exclude the influence of the surrounding plots, only the two rows in the middle of each plot were used for observation. For the visual assessment the potato plant was divided into three leaf levels (lower, middle, upper leaf level) in order to follow disease development. One leaflet per

leaf level was examined to determine the percentage of necrotic leaf area. This rating was done by using a scheme for evaluation of the leaf necrosis in percentage from Granovsky and Peterson (1954).

## RESULTS AND DISCUSSION

In all three stages of sensitivity testing (*in vitro*, *in vivo* and in the field) there was a clear tendency that SDH mutations in *A. solani* have a negative effect on the sensitivity to SDHI-fungicides (Tab.1).

**Table 1.** Overview of different sensitivity factors regarding SDH- wildtype and -mutant isolates (means). n=number of isolates. \*rAUDPC of untreated control in the wildtype inoculation: 0,49; in the mutant-inoculation: 0,57. In this table, only the results for the variety Lady Amarilla are described.

	SDH-wildtype isolates	SDH-mutated isolates
<i>In vitro</i> (EC50 values with Cantus)	0,1µg/ml (n=9)	154 µg/ml (n=20)
<i>In vivo</i> (Fungicide-efficacy with boscalid)	99% (n=3)	69% (n=6)
In the field (rAUDPC)* (with Cantus)	0,37 (n=1)	0,49 (n=2)

Furthermore, the detected fungicide efficacy in the field differed between the two mutant inoculations, although they have the same mutation C-H134R.

In addition, in this first field trial, the four different SDHI-fungicides, which were used for the experiment, also showed a variability in the fungicide-efficacy both for the wildtype and the mutant inoculation.

## CONCLUSION

*In vitro*, *in vivo* and in the field, a reduced SDHI-fungicide efficacy was observed. To confirm these results, especially from the annual field trial, a second (and third) field experiment needs to be done.

It was also observed in the annual field trial that even if there's a decreasing SDHI-fungicide efficacy, there is a high dependency on the active ingredient of each fungicide.

All in all, the three stages of sensitivity testing give a deeper knowledge about the raising loss of fungicide-efficacy and the epidemiology of the pathogen.

Due to the fact, that mutations in the Succinate dehydrogenase of *A. solani* lead to a decreasing fungicide-efficacy even in the field and it is important to get a deeper knowledge about the efficacy of different SDHIs regarding the increasing and changing SDHI mutant population in European potato growing areas.

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