TECHNISCHE UNIVERSITÄT MÜNCHEN

TUM School of Life Sciences

Lehrstuhl für Phytopathologie

ENVIRONMENT INTERACTION, FUNGICIDE RESISTANCE AND BIOLOGICAL CONTROL OF *ALTERNARIA SOLANI* ON POTATO

Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München genehmigten Dissertation zur Erlangung des akademischen Grades eines

Doktors der Agrarwissenschaften

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Diese Dissertation wurde am 23.11.2020 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 19.03.2021 angenommen.

1. Introduction

1.1. Early blight on potato

Early blight on potato in Germany is mainly caused by the fungus *Alternaria solani* Sorauer, 1896. However, several studies showed other large-spored *Alternaria* spp. to be involved in the early blight infection of potato. In Brazil, *A. grandis* Simmons was reported to be the causal agent of early blight (Rodrigues et al. 2010), whereas in Algeria it was found to be *A. protenta* (Ayad et al. 2017). Landschoot et al. (2017b) detected a complex of large-spored *Alternaria* spp. – *A. protenta*, *A. grandis* and *A. solani* – on infected potato leaves in Belgium. The small-spored *A. alternata* can also frequently be found on infected leaves, but it is considered not to cause severe disease (Leiminger et al. 2014). Reported yield losses caused by early blight range from 2 to 60% (van der Waals et al. 2003; Horsfield et al. 2010; Leiminger and Hausladen 2014), depending on environmental conditions as well as plant cultivation aspects, e.g., crop rotation, tillage or cultivar.

1.2. Epidemiology of Alternaria solani

The disease cycle of *A. solani* consists of four main phases during the season: Overwintering, Conidia formation, primary infection and infection of the whole plant.

During winter, *A. solani* survives on infected plant debris or in soil as mycelium or conidia (Rotem 1994). The brown to black conidia are obclavate to elongate shaped and grow individually or in small groups (Rotem 1994). Chlamydospores could also be a source for soil-borne inoculum (Basu 1971; Patterson 1991).

In spring, spore production is induced at temperatures between 5 and 30°C and favored by alternating periods of wet and dry conditions (Pscheidt 1985). Spores are dispersed by wind, insects, or rain splash from the ground on to the leaves near the ground (Rotem 1994). Spore germination is dependent on the presence of free moisture (from rain, dew, fog, irrigation). Favorable temperature for infection is between 20 and 30°C, but a minimum temperature of 10°C can be sufficient, if leaf wetness and inoculum density are ideal for the pathogen (Waggoner and Horsfall 1969). Penetration of plant tissue is possible either directly or through wounds and stomata. Typical symptoms are dark brown to black lesions with concentric rings and a chlorotic halo, which first appear on physiologically older leaves (Rotem 1994). Day-night fluctuations in temperature, radiation and leaf moisture are the reasons for this target-like pattern, and therefore these characteristic rings are missing in greenhouse trials. The delivery of produced toxins to neighboring cells induces the surrounding chlorotic halo (Pscheidt 1985). After the primary infection, the pathogen has a high capacity to produce secondary inoculum in multiple cycles during the growing season (Campo et al. 2007). Harrison et al. (1965) observed a strong increase of *A. solani* spores in the air during the season, which supports the distribution of conidia via wind to neighboring plants. By time, lesions expand and fuse to big necrotic areas, which can finally lead to the death and abscission of the leaf (Pscheidt 1985). During harvest, also potato tubers are raley infected with *A. solani*. Sunken, dark and irregular lesions on the surface characterize this dry rot on tubers.

1.3. Secondary metabolites of A. solani

A. solani produces several different toxins (Tab. 1). The ability to produce toxins may contribute to pathogenicity as suggested by Yoder (1980).

Table 1 Most frequently produced secondary metabolites by *A. solani*.

Metabolites	**Reference**
Alternariol (AOH)	Lee and Yu 1995
Alternariol methyl ether (AME)	Lee and Yu 1995
Alterporriol	Pinto and Patriarca 2017
Altersolanol A	Andersen et al. 2008
Altertoxin	Andersen et al. 2008
Macroscopin	Andersen et al. 2008
Zinniol	Cotty and Misaghi 1984

1.4. Control of early blight

In the field, several options are available for the control of early blight. To minimize the risk of an infection at the beginning of the season, the amount of primary inoculum and the plant nutrition level play an important role. Upon the fungal is established in the field, chemical and biological control agents can be used during the season according to good agricultural practice.

1.4.1. Nutrition levels

An ideal supply of nutrients, especially of nitrogen, is a prerequisite for a healthy plant. It was shown that nitrogen is a key component for preventing early blight infections. Increasing amounts of nitrogen stepwise reduce *A. solani* infection in the field (Barclay et al. 1973; Soltanpour and Harrison 1974; MacKenzie 1981; Miller and Rosen 2005; Mittelstrass et al. 2006). Besides the total amount of nitrogen, the timing of application is also important to optimize the influence on early blight susceptibility (Abuley et al. 2019). Mittelstrass et al. (2006) also observed that early blight infection is affected by decreasing amounts of nitrogen to a higher degree when compared to late blight. The mechanism behind this increasing resistance is not fully understood yet. Mittelstrass et al. (2006) discussed the delayed protein degradation as possible reason for the increased resistance to *A. solani*, because they observed higher protein contents in plants with higher N fertilization. Rotem (1994) assumed that the increasing amount of soluble sugars can inhibit spore germination of *A. solani* and indeed, Mittelstrass et al. (2006) also found a higher sucrose content in the high N variant. Besides, the concentration of potato alkaloids, such as solanine or chaconine, might also be influenced by the N supply of the plant and some of these alkaloids are also discussed as resistance factors (Sinden et al. 1973). Especially during drought periods, foliar fertilizer should be considered, because the uptake of nitrogen from the soil is less efficient than under normal conditions (Dalla et al. 1997). Regarding phosphate, by contrast, a decreasing supply leads to reduced susceptibility to early blight (Barclay et al. 1973).

1.4.2. Phytosanitary aspects

A. solani is overwintering as mycelia or conidia in plant debris and in the soil (Pscheidt 1985). Therefore, it is essential to reduce the soil-borne inoculum to a minimum. Crop rotation is one of the

critical components: short crop rotations with frequent cultivation of host-plants (potato, tomato) increases early blight infections (Shtienberg and Fry 1990). In addition to the apparent crop plants, other non-typical host-plants like black nightshade (*Solanum nigrum L.*) or field thistles (C*irsium arvense*) also need to be controlled to minimize inoculum levels.

Several studies observed a disease-reducing effect of soil treatments (fumigation, cropping systems) (Harrison et al. 1965; Basu 1974; McCarter et al. 1976; Olanya et al. 2009). These findings support the assumption that the main source for primary infection is soil-borne inoculum.

1.4.3. Cultivar and Seeds

Potato cultivars from the middle to late maturity group are, in general, less susceptible to early blight compared to early maturing cultivars (Douglas and Pavek 1972; Johanson and Thurston 1990; Abuley et al. 2018). However, in each maturity group, a broad range of more and less susceptible cultivars was observed (Douglas and Pavek 1972; Leiminger and Hausladen 2014).

1.4.4. Chemical control agents and resistance against them

In many cases, potato early blight disease pressure gets too strong, and the farmers need to spray chemical fungicides to protect the yield. In general, three different fungicide groups are currently available for specific control of early blight: Quinone-outside inhibitors (QoIs), Succinate dehydrogenase inhibitors (SDHIs) and Demethylation inhibitors (DMIs). Because each of the three fungicide groups has only one specific mode of action, there is a high risk for fungicide resistance development (Kuck and Mehl 2003).

The QoIs bind to the cytochrome b (*cytb*) and inhibit electron transport between *cytb* and cytochrome c_1 in mitochondrial electron transport complex III (Bartlett et al. 2002). QoI binding leads to a blocking of the production of ATP, with the consequence that the fungus lacks energy. In *A. solani,* one mutation in *cytb* is already widespread, namely the F129L mutation (Pasche et al. 2004; Leiminger et al. 2014; Odilbekov et al. 2016). This mutation leads to a reduced sensitivity of the pathogen to QoIs, but not to a complete loss of fungicide efficacy.

Similar to the QoIs, the SDHIs also interfere with the mitochondrial respiration, but at mitochondrial electron transport complex II (Kuhn 1984). There, they inhibit the enzyme succinate dehydrogenase. The complex II consists of four *Sdh* subunits and the SDHIs bind to three of them: *SdhB*, *SdhC* and *SdhD* (Horsefield et al. 2006). Mallik et al. (2014) described five point mutations in the context of SDHI resistance– two in the *SdhB* subunit, one in the *SdhC* subunit, and two in the *SdhD* subunit. In detail, the amino acid exchange from histidine at the position 278 to arginine or tyrosine in the subunit B leads to the *SdhB*-H278R and *SdhB*-H278Y mutations, respectively. In subunit C, the mutation *SdhC*-H134R is caused by a substitution of histidine to arginine at position 134. A similar amino acid exchange in subunit D at location 133 leads to the *SdhD*-H133R mutation. Additionally, a replacement of aspartate to glutamic acid at position 123 generates the *SdhD*-D123E mutation. These mutations can cause a reduced fungicide efficacy (Gudmestad et al. 2013; Landschoot et al. 2017a; Metz et al. 2019). In 2014, Mallik et al. (2014) and later also Landschoot et al. (2017a) detected the first *Sdh*-gene double mutants.

The DMIs inhibit the C-14-α-demethylase in the sterol biosynthesis pathway. The triazoles represent the most common chemical structures within the DMIs. For DMI resistance, no mutations in *A. solani* are known so far. But, if we have a look into other related pathogens like *Parastagonospora nodorum* or *Zymoseptoria tritici*, several mutations in the 14α-demethylase (CYP51) gene are described, that result in reduced fungicide sensitivity (Stammler et al. 2012; Pereira et al. 2017; Blake et al. 2018). Beside mutations in this gene, an overexpression of the CYP51 gene can also promote pathogen resistance like it is described for *Penicillium digitatum*, *Venturia inaequalis* or *Blumeriella jaapii* (Hamamoto et al. 2000; Schnabel and Jones 2001; Ma et al. 2006). Hayashi et al. (2003) and Reimann and Deising (2005) observed another common mechanism to reduce sensitivity to DMIs in *Pyrenophora tritici-repentis* and *Botrytis cinerea*: the activation of energy-dependent fungicide efflux transporters.

1.4.5. Biological Control Agents (BCAs)

There're not many studies about the use of biologicals within the pathosystem of *A. solani* and potato. El Gamal et al. (2018) was able to show the antagonistic effects of *Chaetomium globosum* and *Trichoderma harzianum* against *A. solani* of potato in dual culture tests.

In the comparable pathosystem of *A. solani* and tomato, studies with *Bacillus subtilis* or plant growth-promoting rhizobacteria (PGPR) illustrate the positive effect of biological control agents on the reduction of early blight infection *in vivo* (Awan and Shoaib 2019; Shoaib et al. 2019; Attia et al. 2020). Song et al. (2015) showed that an arbuscular mycorrhizal fungus could enhance plant resistance against early blight in tomato. Another focus lies on the fungal genus *Trichoderma*. Several studies based on *in vitro* dual-culture tests of *Trichoderma* spp. with *A. solani* are published. Lakhdari et al. (2018) and Mazrou et al. (2020) recorded comparable inhibition rates of about 60% with *T. harzianum in vitro*. Consolo et al. (2012) observed a high variation between different *Trichoderma* strains, even from the same species: They displayed between 17 and 63% inhibition of *A. solani* growth by diverse strains of *T. harzianum*. Other studies also show the ability of *Trichoderma* spp. to reduce infection of *A. solani* on intact tomato plants (Fontenelle et al. 2011).

Various studies with *A. solani* from unknown hosts also described a broad range of interactions between possible Biological Control Agents (BCAs) and the pathogen. On the one hand, inhibiting effects of metabolites from *Diaporthe eucalyptorum* or of extracts from various plants on *A. solani* were observed *in vitro* (Onaran 2016; Gao et al. 2020). On the other hand, studies with living organisms like *Bacillus veleszensis* or *Trichoderma* spp. were conducted in dual culture tests and verify their high potential as BCAs (Prabhakaran et al. 2015; Grady et al. 2019; Idrees et al. 2019; Mazrou et al. 2020).

1.5. Trichoderma spp.

The genus *Trichoderma* is classified as *Ascomycota* and belongs to the family of *Hypocreaceae*. It can be found in nearly all types of temperate and tropical soils (Samuels 2006) and is a ubiquitous colonizer of cellulosic materials (Kubicek et al. 2008). Besides the industrial use of *T. reesei* for the production of cellulase, the ability of *Trichoderma* as a biocontrol agent is of rising interest since the pioneering work of Weindling (1932). Several reviews on the mechanisms and the potential use of *Trichoderma* were published, which show the importance and the potential of this BCA (Papavizas

1985; Howell 2003; Benítez et al. 2004; Harman 2006; Verma et al. 2007; Vinale et al. 2008; Adnan et al. 2019).

Trichoderma offers a whole range of biocontrol mechanisms: Competition, antibiosis, mycoparasitism, induction of resistance, and it can serve as a source of specific genes for biotechnological approaches. This diversity makes *Trichoderma* a perfect candidate for a BCA to study in more detail.

1.5.1. Competition

Trichoderma has to compete with other fungi about space and nutrients in the soil. Because of its relatively fast growth, *Trichoderma* is able to colonize soils very successfully. Because starvation is one of the most common reasons for microorganisms to die – and especially iron is essential for most filamentous fungi – the highly efficient iron uptake of *Trichoderma* is a big advantage and supports outcompeting other fungi (Chet and Inbar 1994).

1.5.2. Antibiosis

Trichoderma produces various secondary metabolites. Mainly low-molecular-weight diffusible compounds (including volatile organic compounds (VOCs)) and antibiotics are involved in the inhibition of other macro- or microorganisms. For many of these metabolites, antibiotic activity has been described (Alain et al. 2001). Weindling (1934) observed the antifungal activity of the first *Trichoderma*-derived metabolite gliotoxin and its potential role in the antagonistic activity. The volatile compound 6-pentyl-α-pyrone was shown to inhibit mycelial growth of *Pythium ultimum in vitro* (Vinale et al. 2008) and can induce changes in leave metabolome composition (Mazzei et al. 2016). Besides the complexity of the function of the single compounds, also synergistic effects between *Trichoderma* metabolites occur (Lorito et al. 1994; Lorito et al. 1996).

1.5.3. Mycoparasitism

The process of mycoparasitism starts with the sensing of the host fungus. It is assumed that *Trichoderma* continuously releases a small amount of exochitinases into the soil. These enzymes diffuse to host fungi and initiate a release of fragments of their cell wall. Sensing of those chitin fragments leads to the expression and diffusion of more cell wall degrading enzymes (CWDE) from *Trichoderma* to attack the host fungus (Brunner et al. 2003). This whole process takes place before direct contact between *Trichoderma* and its host fungi (Zeilinger et al. 1999). With this initiation, *Trichoderma* facilitates its growth and directly attacks the host fungus, including hyphal coiling, penetration and finally killing the target organism (Rabea et al. 2003).

1.5.4. Induction of localized and systemic resistance

In 1997, Bigirimana et al. showed the ability of *Trichoderma* to induced resistance in plants. Many studies targeting induced resistance by *Trichoderma* have been carried out since then. The endophytic activity of *Trichoderma* has been detected and several strains can colonize roots (Thrane et al. 1997; Metcalf and Wilson 2001). The penetration of root tissue is mostly limited to the first and second layer of cells (Yedidia et al. 1999; Metcalf and Wilson 2001). In this zone of interaction, *Trichoderma* releases secondary metabolites, homologues of avirulence (Avr) proteins or CWDEs (Harman et al. 2004). In turn, the defragmentation of plant cell wall enhances resistance responses in

the plants. Biochemical factors are released, which limit the growth of *Trichoderma*. The same or similar biochemical factors may now also inhibit the growth of pathogens, which try to attack the roots (Harman et al. 2004). Also, several strains induce resistance on leaves, even though they are applied to roots. Some *Trichoderma* strains can induce metabolic changes in plants, which lead to more resistance against a variety of pathogens. Yedidia et al. (1999) showed increased peroxidase and chitinase activity in leaves and roots after root treatment. Mazzei et al. (2016) measured enhanced contents of acetylcholine and γ-aminobutyric acid in tomato leaves after seed treatment with secondary metabolites of *Trichoderma* strains.

1.5.5. Transgenic plants

The genus *Trichoderma* not only serves as a BCA, but also as a rich source of genes to generate more resistant crop plants with transgenic approaches. Several studies proofed the successful implementation of *Trichoderma* genes into potato, tobacco, *Arabidopsis*, and other plants to enhance resistance towards pathogenic fungi like *A. alternata*, *A. solani*, *R. solani* or *Botrytis cinerea* (Lorito et al. 1998; las Mercedes et al. 2006; Montero-Barrientos et al. 2010).

1.6. Objectives

This study aims to get more profound knowledge about the epidemiology of early blight on potato and to improve the integrated disease management. Besides epidemiological studies and the interaction with the environment, the resistance development against one of the mainly used fungicide groups – the Succinate Dehydrogenase Inhibitors (SDHIs) – and its impact on the pathogen and the disease control is evaluated from *in vitro* to field studies. In addition to chemical plant protection products, the ability of *Trichoderma* spp. as Biological Control Agent (BCA) is demonstrated in several testing systems.

2. Material and Methods

2.1. A. solani and Trichoderma spp. isolates

All *A. solani* isolates, which are used for the following studies, are isolated from naturally infected plant tissue.

The *Trichoderma* strains were provided from the University of Szeged, Hungary and listed in table 2. The isolates were gained and published by Körmöczi et al. (2013).

Table 2 *Trichoderma* strains

Isolate number	*Trichoderma* species	Origin	GenBank accession number of ITS
20761	T. *harzianum*	Tomato	JX173832
20770	T. *harzianum*	Tomato	JX173856
20780	T. *atroviride*	Tomato	JX173860
20781	T. *atroviride*	Tomato	JX173866
20784	T. *hamatum*	Carrot	JX173868
20866	T. *asperellum*	Parsley	JX173862

2.2. Media

Depending on fungal and purpose, different types of media were used for cultivation. SNA medium was used for general cultivation and a focus on spore production of *A. solani* (Tab. 3). To promote mycelium growth of *A. solani*, the fungal was cultivated on V8-agar (Tab. 4).

¼ PDA medium was mainly used for the cultivation of *Trichoderma* spp. (Tab. 5).

A specific liquid medium was used to cultivate *Alternaria* in liquid culture for the evaluation of toxin production (Tab. 6).

SNA:

Table 3 Recipe SNA medium for 1L

Substance	Amount
KH_2PO_4	1 g
KNO_3	1 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
Agar	22 g
1 M NaOH	600 µl
H_2O dest.	1 l

V8-Agar:

Table 4 Recipe V8-agar for 1L

Substance	**Amount**
Agar	15 g
$CaCO_3$	2 g
Lindauer-Gemüsesaft	200 ml
H_2O dest.	800 ml

¼ PDA:

Table 5 Recipe ¼ PDA media

Substance	**Amount (¼ PDA)**
Agar	15 g
Potato Extract Glucose Agar	9.75 g
H_2O dest.	1 l

Liquid medium for *Alternaria* cultivation in liquid culture

Table 6 Recipe for liquid cultivation of *Alternaria* (according to Gotthardt et al. (2020))

Substance	**Initial weight [mg]**	**Volume stock solution [mL]**	**Concentration of stock solution [g/L]**	**Volume for one sample [µl]**
KH_2PO_4	625	1000	0.625	20.000
KCl	312.5		0.3125	
$MgSO_4$x 7 H_2O	312.5		0.3125	
$FeSO_4$ x 7 H_2O	25.52		0.02552	
$(NH_4)_2SO_4$	300	7.5	40	125
$NaNO_3$	3003	20	150.15	333
$Ca(NO_3)_2$ x $4H_2O$	600	4	150	50
Glucose	5760	14.4	400	250
NaOAc x 3 H_2O	2322.3	3.5	400	25

pH= 5.5

2.3. Isolation and identification of A. solani strains

For the monitoring or re-isolation from artificially inoculated plants, I gained *A. solani* spores from infected leaves to extract the DNA and sequence for *Sdh*-mutations.

2.3.1. Single spore isolation from infected leaves

After drying, the leaves with specific *Alternaria* symptoms were surface-sterilized for 3 minutes in NaOCl (3%), washed with sterile dest. H_2O for another 3 minutes and then placed with the upper side on SNA plates. To promote fungal growth, the plates were incubated under near UV light (12h/12h) and 21°C for 5 to 7 days.

After the incubation time, a single spore can be picked from the grown fungal with the help of a binocular (Zeiss, 40x magnification) and an injection needle under sterile conditions and placed on a new SNA plate. The single spore isolates were then incubated again under near UV light (12/12h), and 21°C until the petri dish is entirely overgrown and ready for further use and storage.

Besides the storage of single spore isolates as an entirely overgrown SNA plate, the strains were also grown on SNA plates with filter paper (Sartorius 1289, Ø80mm). When the filter paper is fully covered, it can be ripped into small pieces, collected in a tube and frozen in -20°C for long term storage.

To make sure that the large spores, which were grown out of the typical early blight symptoms, were *A. solani*, some random isolates were analyzed via PCR (according to Landschoot et al. (2017b)).

2.3.2. DNA extraction

For DNA extraction, I used a cetyl trimethyl ammonium bromide protocol (CTAB). *A. solani* isolates were grown on V8-agar for two weeks. Afterwards, max. 250mg of mycelia were scratched off with a scoop and transferred to a 2ml tube and put in the freezer. I added glass beads to the samples and dipped all tubes in liquid nitrogen before using the Tissue lyser for three minutes at 30Hz to support optimum grinding. Next 1ml CTAB-buffer (2% CTAB, 100mM Tris (pH 8), 20mM EDTA (pH8), 1.4mM NaCl) was added to each sample, homogenized by vortexing and incubated at 60°C for 60 minutes in a water bath or heating block. After cooling down, 1ml Chloroform:Isoamyl alcohol (49:1) was added and shaken strongly for 20 minutes in an invert shaker (Sunlab). The solution was centrifuged with 5000 rpm for 10 minutes and the supernatant was transferred to a new tube. After adding 750µl of isopropanol, the samples were inverted gently and centrifuged at 13000 rpm for 5 minutes. The isopropanol was discard and the DNA pellet stuck to the bottom of the tube. The pellet was washed with 1ml of cold ethanol (70%). Afterwards, the ethanol was removed completely, and the pellet was dried in a heating block at 37°C for 10-30 minutes. The dry DNA pellet was then dissolved in 100µl distilled H_2O, 5µl of RNAseA (10mg/ml) were added and incubated for 60 minutes at 60°C.

I measured the DNA quality and quantity with the NanoDrop ND1000 (PeqLab).

2.3.3. Sequencing for Qo- and *Sdh*-region

The genomic DNA, which was extracted using the CTAB-protocol, was adjusted to 20ng/µl for *SdhC*/*D* screening and to 40ng/µl for *SdhB*. The used primers are listed in table 7.

For PCR of *SdhC* and –*D* region (570 and 607 bp respectively), 25µl of a reaction mixture containing 40ng DNA, 1.5mM 10x buffer (15mM $MgCl_2$), 0.6µM forward and reverse primer each, 2mM dNTPs and 1U Taq-polymerase was used.

For PCR of *SdhB* region (1082 bp), 50µl of a reaction mixture containing 80ng DNA, 1.5mM 10x buffer (15mM $MgCl_2$), 0.6µM forward and reverse primer each, 2mM dNTPs and 1U Taq-polymerase was used.

The reaction mixture for the Qo-region (214 bp) contained 10xPCR buffer, 1.5mM $MgCl_2$, 200µM dNTP, 0.6µM forward and reverse primer each, 1U Taq-polymerase and 50ng DNA. Total volume: 25µl.

Table 7 Primer sets for detection of succinate dehydrogenase (SDH) and quinone outside inhibitor (QoI) mutations in *A. solani* isolates by PCR (Metz et al. 2019)

Target	Species	Primer name	Primer sequence	References
SdhB	*A.solani*	SdhB-F SdhB-R	ATGGCCTCCATACGCGCTTT CTAGGTGAAGGCCATGCTCTT	Mallik et al. (2014)
SdhC	*A.solani*	SdhC-F1 SdhC-R2	ATGGCTTCTCAGCGGGTATTTCAGC TCCATCCAGTGCGGATAACC	Mallik et al. (2014)
SdhD	*A.solani*	SdhD-F1 SdhD-R2	ATGGCCTCCGTCATGCGT CCTCGGTGATACCAACATCGTTTGTC	Mallik et al. (2014)
QoI	*A.solani* (genotype I)	As-Gf As-Gr	CGGGGACTAATATTTTGATA TGTTATTTAACCAAGAATGAAA	Leiminger et al. (2014)

Cycling conditions for *SdhB* region:

Task	Temperature (°C)	Time	
Denaturation	95	2min	
Denaturation	95	30s	x30
Annealing	58	30s	
Elongation	72	1min	
Final step	72	7min	

Cycling conditions for *SdhC* and-*D* region:

Task	Temperature (°C)	Time	
Denaturation	95	2min	
Denaturation	95	30s	
Annealing	58	30s	x30
Elongation	72	1min	
Final step	72	45s	

Cycling conditions Qo

Task	Temperature (°C)	Time	
Denaturation	95	10min	
Denaturation	95	1min	
Annealing	54	30s	x31
Elongation	72	30s	
Final step	72	10min	

Afterwards, the PCR products were purified via gel electrophoresis and finally sent for sequencing to LGC Genomics GmbH (Germany).

2.3.4. Monitoring

To monitor the distribution of *Sdh* mutated isolates, we collected and analyzed infected potato leaves from different locations in Germany. In total, single spore isolates from 22, 28, 34 and 26 sites were cultured between 2013 and 2016, respectively. For each location, we obtained up to 15 isolates and sequenced them for their *Sdh*-region. Depending on the participation of farmers and public agencies in the monitoring program, the sites varied from year to year, and no field was sampled twice.

2.4. Epidemiological studies

To get more profound knowledge about the epidemiology of early blight and its causal agent *A. solani*, I analyzed several aspects: latent period, influence of different periods of leaf moisture on *A. solani* infection, impact of rising temperatures, and pathogenicity of *A. solani* strains isolated from non-host plants.

2.4.1. Latent period

I conducted greenhouse trials to determine the latent period of *A. solani* isolates. Therefore, 6-week old plants were cut to three uniformly sized leaflets to have similar primary plants. The well-established cultivar Kuras was used for all trials, because it is known to be susceptible to early blight infections. For this particular trial, I included ten different *A. solani* isolates.

***A. solani* infection assay in greenhouse**

The spore solution of the tested *A. solani* isolates were gained from three-week-old SNA-plates, which were cultivated under near UV-light (12h/12h) and 21°C. Spores were carefully scratched off the plates with distilled water (1% Tween) and an object slide. To avoid later plugging of the chromatography sprayer by remaining mycelium, I filtered the solution through a folded gauze bandage. Afterwards, the spore density of each isolate was assessed and adjusted to 5 $x10^4$ spores/ml. For infection assays, the spore solution was then sprayed on the upper side of the leaflets with a chromatography sprayer until runoff, to evenly inoculate the whole plant. After inoculation, the plants were transferred to infection tents with 20°C and an air humidifier to generate nearly 100% relative humidity for 48 h. A high-pressure mist blower was continuously running for the first two days, and subsequently only started automatically if the air humidity was lower than 70%.

Assessment of latent period

For the determination of the latent period, plants were rated one, two, five and seven days after inoculation (dai) for the occurrence of first early blight symptoms and the disease progression. In the case of assessing the latent period, not only data from specific latent period trials were used for evaluation of this parameter. In various greenhouse experiments, an assessment of disease development 1 and 2 dai was included, to get additional information about the latent period in each trial.

2.4.2. Leaf moisture

I analyzed the influence of leaf moisture duration on *A. solani* infection on potato in greenhouse trials with 6-week old plants from the cultivar Kuras. In total, I included nine different periods of leaf moisture duration in this experiment: 6h, 8h, 10h, 12h, 16h, 18h, 20h, 22h, and 24h. Inoculation with *A. solani* was done like it is described in chapter 2.4.1 with three different *A. solani* isolates and a spore density of $2.5x10^4$ spores/ml. For each time point and *A. solani* isolate, three replicate plants were used. After inoculation, the plants were placed in the incubation tent with 100% relative humidity and subsequently taken out after the distinct time points. Disease development was assessed 1, 2, 5, and 7 dai according to the scheme of Granovsky and Peterson (1954). In total, the whole setup was independently repeated three times.

2.4.3. Temperature

To assess the impact of rising temperatures on the disease development of *A. solani* in the greenhouse, I performed two biologically independent trials with the cultivar Marabel. For the experiments, I studied the disease progression of two *A. solani* isolates (808_4 and 754_2) under three different temperature conditions. For each strain and temperature, four replicate plants were used. To ensure successful infection with *A. solani*, all plants were placed in an infection tent with 100% relative humidity for the first 30h after inoculation. Afterwards, the plants were transferred to three climate chambers with either 18, 22, or 26°C. Disease severity was assessed 1, 2, 5, and 7 dai according to the scheme of Granovsky and Peterson (1954).

For evaluation, I included all plants with an infection rate of at least 10% after seven days of inoculation (Tab. 8).

Table 8 Number of plants per treatment and repetition with at least 10% infection rate after seven days.

	18°C		22°C		26°C	
***A. Solani* isolate**	**1 rep**	**2 rep**	**1 rep**	**2 rep**	**1 rep**	**2 rep**
754_2	4	3	3	2	4	2
808_4	4	3	4	1	4	2

2.4.4. Weather data analysis

To analyze the impact of changing climatic conditions on leaf wetness, I used hourly data from the weather station "Dienstweiler" in Rhineland Palatinate (www.am.rlp.de) from 01.05. to 31.08 for 2016 to 2019, because here leaf wetness is directly measured. For the analysis of the presence of minimum periods of leaf moisture, data was gained from the weather station "Freising" from the Bavarian State Institute for Agriculture (www.wetter-by.de). For this analysis hourly data from 01.05. to 31.08 for 2016 to 2019 was used. The weather station "Freising" is located about 1km linear distance away from our field trials. The altitude of both weather stations "Dienstweiler" and "Freising" is on a similar level (about 450m).

2.4.5. Pathogenicity of *A. solani* strains from non-host plants

For this experiment, I tested 15 *A. solani* isolates from various hosts for their pathogenicity on potato plants (Tab. 9). In detail, we tested isolates from thistle, black nightshade, wild tomato, cultivated tomato, potato tubers, and as a reference from potato leaves. All isolates were gained from naturally infected leaves/tubers. For each strain, three replicate plants were used. Gaining of spore solutions, inoculation and incubation of the plants were done as described in chapter 2.4.1. The spore density for all isolates was $5.4x10^4$ spores/ml. The disease progression was evaluated 1, 2, 5, and 7 dai according to the scheme of Granovsky and Peterson (1954). The whole experiment was independently repeated three times.

Table 9 *A. solani* isolates from different plant species.

Isolate number	Host
803_1	Potato Leaf
1121_5	Potato Leaf
1123_8	Potato Leaf
1134_1	Potato tuber
1134_2	Potato tuber
1134_3	Potato tuber
1118_3	Tomato
1118_5	Tomato
1118_6	Tomato

Isolate number	Host
1132_1	Wild tomato
1132_2	Wild tomato
1116_6	Black nightshade
1075_1	Thistle
1075_2	Thistle
1075_3	Thistle

2.5. SDHI fungicide sensitivity testing

I assessed the sensitivity of single *A. solani* strains against active ingredients, or formulated fungicides in three steps to get a broader picture of SDHI fungicide sensitivity: in the laboratory, in the greenhouse, and in the field.

2.5.1. EC_{50} value *in vitro*

To assess the sensitivity of *A. solani* isolates against either active ingredients or formulated fungicides, I performed an *in vitro* plate assay in the first step. This assay is based on the germination of conidia on fungicide amended agar (Olaya et al. 1998). To determine the EC_{50} value, which describes the fungicide concentration that effectively reduces germination by 50% relative to the untreated control, we used five different levels: 0, 0.1, 1.0, 10.0, and 100.0 µg active ingredient/ml.

This assay was performed with two fungicides, which both belong to the group of succinate dehydrogenase inhibitors: Cantus® (active ingredient boscalid) and Luna® Privilege (active ingredient fluopyram).

For the active ingredient boscalid, we had trouble dissolving it at higher concentrations, so the commercial product Cantus® (BASF; 500g boscalid/l) was used. For the highest concentration of the active ingredient, Cantus® was dissolved in distilled H_2O to a final concentration of 100 µg boscalid/ml in the agar. A stock solution was prepared for the lower levels and added to the medium in different amounts. For each experiment, a new stock solution was used to exclude degradation of compounds. The same procedure was done with the active ingredient fluopyram. To ensure comparability between both substances, the commercial product Luna® Privilege (Bayer; 500g fluopyram/l) was used instead of the active ingredient itself. The diluted compounds were added to 1.5% water agar after autoclaving (121°C, 20 min). The sensitivity was determined by comparing spore germination on 1.5% water agar with a concentration of 0, 0.1, 1.0, 10.0, and 100.0 µg/ml active ingredient (boscalid or fluopyram).

To assess the germination rate of each *A. solani* isolate, I obtained a spore suspension from 14 days old SNA plates, which were cultivated under near UV light (12h/12h) and 21°C. The conidia were scratched off with a sterile object slide and distilled water with 1% Tween20 (MERCK). The conidial suspension was adjusted to 1 x 10^4 conidia/ml using a haemocytometer. 130 µl of the suspension of each isolate was added to two replicate plates of each fungicide dilution. To ensure even distribution of the conidia on the plates, a sterile drigalski spatula was used. Germination of 100 conidia was then assessed after five hours of incubation in darkness at 26°C, by using a binocular microscope at x40 magnification. A conidium was considered as germinated if the germ tube was at least half the length of the conidium. The EC_{50} value was determined for each isolate, according to Pasche et al. (2004). This value describes the fungicide concentration that effectively reduces germination by 50% relative to the untreated control. The calculated EC_{50} values for the boscalid-sensitivity screening were divided into three groups, according to Gudmestad et al. (2013): sensitive (<5µg/ml), moderately resistant (5 to 20µg/ml) and highly resistant (>20 µg/ml). For the subset of isolates, which were tested both on boscalid and fluopyram sensitivity, the calculated EC_{50} values were categorized into four separate groups. The categorization for boscalid was: sensitive (<5µg/ml), moderately resistant

(5 to 20µg/ml), very resistant (20-100µg/ml) and highly resistant (>100µg/ml) and for fluopyram: <0.1µg/ml, 0.1-1µg/ml, 1-5µg/ml and >5µg/ml.

2.5.2. Fungicide efficacy in greenhouse

For greenhouse trials, 6-week-old plants were cut to three uniformly sized leaflets and all the other leaves were removed to have similar plants for all tests. We used the potato cultivar Kuras, because this cultivar is known to be susceptible to early blight.

One day before inoculation with the pathogen, I sprayed the plants preventively with different fungicide concentrations. The fungicide efficacy in greenhouse was assessed for boscalid with concentrations of 0.1, 1.0, 10.0, and 100.0 µg/ml. An untreated, but *A. solani*-infected control and an untreated non-infected control were always included in the trial. Three biological replicate plants per treatment were used. One day before the inoculation with *A. solani*, the solutions with the different amounts of boscalid were applied until runoff, to wet the whole plant evenly.

The next day, I infected the plants with *A. solani,* as described in 2.4.1. The spore density was between 6.8 and 10.0 x10^4 spores per ml.

The whole experiment was repeated twice with all five concentrations and once only with the highest dose of boscalid (100.0 µg/ml). For the final analysis, the experimental data from 100.0 µg/ml boscalid treatment was used, as this concentration showed the highest differentiation. To determine the disease severity, I visually assessed the percentage of infected leaf area according to the scheme of Granovsky and Peterson (1954) for each of the three leaflets. Disease progression was rated 5, 7, and 10 days after inoculation.

2.5.3. Fungicide sensitivity in the field

To get a complete picture about the influence of *sdh* mutations on the fungicide efficacy, I also conducted field trials. Therefore, we designed a trial setup with artificial inoculation, to observe direct interaction between a prevalence of *sdh* mutated isolates vs. *sdh*-wild type isolates in the field and SDHI fungicide treatments over the whole season.

Experimental design

The field trials were located in Weihenstephan, Germany, in 2016, 2017, and 2018. Basic parameters are displayed in table 10. Each year a different field was used to avoid potato crop after potato crop and to keep the soil-born inoculum as low as possible. We used a randomized design with four replicate plots per treatment for all our field trials. For the fungicide sensitivity testing, we used the potato cultivars Lady Amarilla from the early maturity group and Maxilla from the middle-late to late maturity group, which are both known to be very susceptible to early blight.

Table 10 Basic parameters for SDHI fungicide sensitivity testing under field conditions.

Year	2016	2017	2018
Location	Weihenstephan	Weihenstephan	Weihenstephan
Soil	Silt-loam	Silt-loam	Silt-loam
Cultivar	Lady Amarilla, Maxilla	Lady Amarilla, Maxilla	Lady Amarilla, Maxilla
Plot size	4x4.5m	4x4.5m	4x4.5m
Planting date	10.05.2016	11.05.2017	11.04.2018
Emergence	12.06.2016	14.06.2017	11.05.2018
Isolates for inoculation	*A. solani* 615 (wild type) *A. solani* 628 (*SdhC*-H134R mutant) *A. solani* 687_1 (*SdhC*-H134R mutant)	*A. solani* 615 (wild type) *A. solani* 628 (*SdhC*-H134R mutant) *A. solani* 687_1 (*SdhC*-H134R mutant)	*A. solani* 615 (wild type) *A. solani* 628 (*SdhC*-H134R mutant) *A. solani* 687_1 (*SdhC*-H134R mutant)
Date of inoculation	29.06.2016	21.06.2017	30.05.2018
Inoculation density	5g infected kernels/m²	5g infected kernels/m²	5g infected kernels/m²
Early blight specific fungicides	Cantus®[1] Luna® Privilege[2] Sercadis[3] Vertisan®[4]	Cantus®[1] Luna® Privilege[2] Sercadis[3] Vertisan®[4] Narita®[5]	Cantus®[1] Luna® Privilege[2] Sercadis[3] Vertisan®[4] Narita®[5]
Number of SDHI treatments	4xLady Amarilla 4xMaxilla	3xLady Amarilla 4xMaxilla	4xLady Amarilla 5xMaxilla

[1]Syngenta (500g boscalid/kg)

[2]Bayer (500g fluopyram/l)

[3]BASF (300g fluxapyroxad/l)

[4]Dupont (200g penthiopyrad/l)

[5]Belchim (250g difenoconazol/l)

Assessment of meteorological data

For the period of potato growth (April to September), I summarized data of average weekly mean air temperature, total weekly rainfall and leaf moisture. Data was gained from the Bavarian State Institute for Agriculture (www.wetter-by.de). The weather station "Freising" is located about 1km linear distance away from our field trials. The data for leaf moisture aren´t directly measured values, but calculated by a model from the Bavarian State Institute for Agriculture.

Artificial inoculation

I carried out a targeted kernel inoculation each year to ensure a predominance of specific *A. solani* isolates on the field. Therefore, three different *A. solani* isolates were used – two *SdhC*-H134R mutants (628 and 687_1) and one *Sdh*-wild type (615). All three strains also carried the F129L mutation in complex II (QoI-mutation).

To generate the infected kernel inoculum, entirely overgrown SNA plates of the three different isolates and bags filled with sterile barley grains were needed. 150g barley grains per bag and 60ml distilled water were prepared and autoclaved twice. For closing the bags, a buckler and rubber band was used. Between first and second autoclaving, the kernels were allowed to cool down and mixed

to support even distribution of water in the bags. The three different *A. solani* isolates were cultivated on SNA plates under near UV-light (12h/12h) for two weeks and 21°C. For infection with *A. solani*, half of an overgrown plate was cut into small pieces and mixed with the kernels. The bags were incubated for four weeks under near UV-light (12h/12h) to support fungal growth. After two weeks, the bags were kneaded again and turned around to ensure an even illumination.

I then spread the infected kernels equally between the potato rows (5g/m²) approximately six weeks after planting. To minimize intermixing of the single isolates by wind or rain splash, the field was divided into four parts, and each part was inoculated with a different strain. The fourth part of the field was used as non-inoculated control. After each section, an additional 4m plot was included as a natural barrier between different *A. solani* inoculations. Within each isolate, a randomized design with four replicates was used.

Fungicide treatments

To test fungicide sensitivity against SDHIs, four SDHI-fungicides with the same mode of action, but different active ingredients were used: Cantus® (500g boscalid/kg), Luna Privilege (500g fluopyram/l), Sercadis (300g Fluxapyroxad/l) and Vertisan (200g Penthiopyrad/l). In 2017 and 2018, the Demethylation inhibitor fungicide Narita (250g Difenoconazol/l) was included as a non-SDHI treatment. An untreated control was also part of each trial. All fungicides were applied every two weeks with a backpack-sprayer and with a comparable amount of active ingredient. The official application rate of Cantus® (boscalid) with 0.5kg/ha served as a standard.

In addition to the early blight specific treatments, the whole trials were independently sprayed with oomycete active fungicides (alternating 0.6l/ha Revus (250 g Mandipropamid/l) and 0.2l/ha Ranman (400g Cyazofamid/l) against late blight (*Phytophthora infestans*) every week. Furthermore, herbicides and insecticides were sprayed on all plots according to good agricultural practice.

Disease assessment in the field

For the assessment of disease progression, 10 plants per plot were rated for their infected leaf area in percentage according to the scheme of Granovsky and Peterson (1954). To get a more detailed picture of the disease development, plants were divided into three leaf levels – upper, middle, lower leaf level. Only the two rows in the middle of each plot were used for rating to exclude the influence of surrounding treatments. The evaluation was always done by the same person and for all four replicate plots. Period of assessment was from the occurrence of first symptoms till the end of August. At the end of the season, all data were used to calculate the area under the disease progress curve (AUDPC), according to Shaner and Finney (1977), for all plots. For our analyses, we then calculated the relative AUDPC (rAUDPC). This value is the calculated AUDPC for a specific plot relative to the AUDPC with disease severity of 100% at each time point.

2.6. Fitness studies

To study the influence of different *Sdh*-mutations on the fitness level, I analyzed several factors: spore density, spore germination, mycelium growth, latent period, and disease development *in vivo* and toxin production in liquid culture.

2.6.1. Spore density

For the assessment of spore density after two (three) weeks, 7 (7) *Sdh*-wild type (WT), 7 (5) *SdhB*-H278Y mutant, and 7 (6) *SdhC*-H134R mutant isolates were tested (Tab. 11). Each strain was grown twice on SNA plates for 21 days under near UV light (12h/12h) and 21°C. After two or three weeks, respectively, the spores of each Petri dish were washed off with three ml autoclaved distilled H_2O and an object slide. One drop of Tween20 was added to the solution to prevent the spores from sticking together. The spore density of each spore solution was finally determined by using a Thoma cell counting chamber. Each solution was counted 10 times. In the end, only plates with a successful growth of the fungal were included in the counting (Tab. 11)

Table 11 Number of successfully growing *A. solani* plates for spore density determination.

***Sdh*-region**	**Number of strains (2 weeks)**	**Total number of plates (2 weeks)**	**Number of strains (3 weeks)**	**Total number of plates (3 weeks)**
Sdh-WT	7	10	7	13
SdhB-H278Y	7	9	5	9
SdhC-H134R	7	9	6	10

2.6.2. Spore germination

To assess spore germination, I used the spore solution from the plates of the spore density determination in 2.6.1. 130µl of spore solution were pipetted on two H_2O-agar (1.5 %) plates each and distributed equally over the whole plate with a drigalski spatula. Then the Petri dishes were put in a heating cabinet with 28°C for 5h in the darkness. After incubation time, we counted how many spores out of 100 were germinating and calculated the germination rate (%). I considered a spore as germinated, when the germ tube was at least half the size of the spore itself.

2.6.3. Mycelium growth

For determination of mycelium growth, a plug of each isolate (12mm diameter), which was grown on SNA for three weeks, was placed in the middle of a V8-agar plate. For cutting the agar plugs, a 12mm-biopsy puncher (Acuderm Inc., USA) was used. In total, 8 *Sdh*-WT, 7 *SdhB*-H278Y, and 6 *SdhC*-H134R isolates were included in this study. Ten V8-agar plates were inoculated for each strain. All Petri dishes were incubated for 14 days under near UV-light (12h/12h) and 21°C. The growth of the isolates was assessed after seven and 14 days post-inoculation. Therefore, a picture was taken from each plate, and afterwards, the area of the growing fungal was calculated using the program ImageJ. In the end, only plates with a successful growth of the fungal were included in the calculations (Tab. 12).

Table 12 Number of successfully growing *A. solani* plates for mycelium growth determination.

***Sdh*-region**	**Number of isolates**	**Number of plates**
Sdh-WT	8	39
SdhB-H278Y	7	40
SdhC-H134R	6	29

2.6.4. Disease development *in vivo*

For most greenhouse experiments, the potato cultivar Kuras, which is highly susceptible to early blight, was used. The six weeks old plants were always cut to three fully developed leaflets, to have the same conditions for all trials and to simplify inoculation and rating. For infection with *A. solani*, the isolates were grown for three weeks under near UV light (12/12) on SNA media to support spore production.

2.6.5. Toxin production

For some *A. solani* isolates, the production of Alternariol and Alternariol Monomethylether was measured in cooperation with the chair of analytical food chemistry, TUM, according to their published protocol (Gotthardt et al. 2019). Therefore, the isolates were cultivated in liquid media, which was optimized for *Alternaria alternata* (Gotthardt et al. 2020). 100ml Erlenmeyer flasks were filled with 25ml of stock solution and then autoclaved at 120°C for 20 min. A spore solution from 14-day old plates was gained for each isolate. The spore density was adjusted to $4.5x10^4$ spores/ml. 25µl of each spore solution was then added to the chilled media. For each strain, four replicate flasks were included.

The inoculated flasks were cultivated on a shaker with 110rpm at 26°C in darkness and 30min. of light per day for one week. Afterwards, the media of each flask was filtered through a gauze bandage and adjusted to a pH of 2. For the following solid-phase extraction, C8 cartridges (Discovery DSC-8, Supelco, Bellefonte, PA, USA) were used. The quantification of the amount of AOH and AME was done via LC-MS/MS in cooperation with M. Rychlik from the chair of analytical food chemistry, TUM, according to Gotthardt et al. (2019). To calculate quantitative values, a one-point calibration was included.

2.7. Trichoderma spp. as biological control agent

To analyze the potential of our *Trichoderma* strains as a biocontrol agent against *A. solani*, I used three different types of studies: Dual culture tests *in vitro*, infection assays in greenhouse and field trials.

2.7.1. Dual culture test

For the dual-culture test, according to Morton and Stroube (1955), I cultivated three different *A. solani* strains on SNA for 14 days and the six *Trichoderma* strains grew on ¼ PDA for one week. For the performance of the test, we decided to use ¼ PDA, which is suitable for both fungi.

Now, one agar plug of each fungal can be placed on opposite positions on the plates. To cut the agar plugs, a 12mm-biopsy puncher (Acuderm Inc., USA) was used. Because *Trichoderma* grows much faster than *A. solani*, we slightly adjusted the method. We decided to put *A. solani* two days earlier on the plate than *Trichoderma* to promote the establishment of *A. solani* and to see a better interaction between both fungi in the end. Petri dishes with either *Trichoderma* or *A. solani* on their own served as controls. The plates were incubated for five days under normal day/night conditions and 20-22°C. For all combinations, five repetitions were included.

Fungal growth was determined by measuring the area of grown mycelium after five days with imageJ. Growth inhibition was calculated with this formula:

$$Growth\ inhibition\ (\%) = \frac{Area\ 5dai\ Treated\ (mm)}{Area\ 5dai\ Control\ (mm)} x100$$

2.7.2. Efficiency in greenhouse trials

For the greenhouse trials, six weeks old potato plants (cultivar Kuras) were used.

To test the efficiency of *Trichoderma* spp. in greenhouse, I made a spore solution of each of the six *Trichoderma* isolates from 14 days old ¼ PDA plates. Therefore, the entirely overgrown plates were scratched off with distilled water and a sterile object slide and filtered through a folded gauze bandage. The spore density was adjusted to $1x10^7$ spores/ml for all strains. The treatment of the plants in greenhouse was carried out one day before the inoculation with *A. solani*. The plants were sprayed with a chromatography sprayer until runoff to ensure even distribution on all leaves. In addition, a fungicide treatment with Bravo® (500g/l chlorothalonil) and two commercial BCAs – Tmix Plus® and TrichoStar® - were included. For each treatment, three replicate plants were used and the whole experiment was repeated twice.

The infection with *A. solani* was conducted one day after *Trichoderma* treatment and was done as described in point 2.5.2. For each *Trichoderma* strain, a control treatment without *A. solani* inoculation was included. In addition, treatments with only *A. solani* and non-treated plants were added as controls.

Further proceeding was similar to greenhouse trials with fungicides, described in point 2.5.2.

2.7.3. Efficiency in field trials

From 2016 to 2019, I carried out four independent field trials in Weihenstephan, Germany. In 2016 and 2018, the cultivar Lady Amarilla and in 2017 and 2019 Maxilla was used (Tab. 13). Similar to the greenhouse trials, I used spore solutions of *Trichoderma* for leaf treatments. Besides the six *Trichoderma* strains tested in each year, two commercial biocontrol products – Tmix plus® (1kg/ha) and TrichoStar® (15l/ha) – were also part of the trials from 2016 to 2018 (Tab. 13). For 2019, they were excluded because I wanted to focus on the pure spore solutions. In the years 2017 and 2018, a mixture of three *Trichoderma* strains was applied, but also excluded again in 2019. In each year, the multisite fungicide Bravo® (3l/ha), with its active ingredient Chlorothalonil, served as a positive control (Tab. 13).

To ensure early blight infection in these trials, kernel inoculation was done each year with *A. solani* 615 (*Sdh*-wild type) and additional pesticide applications were carried out, like it is described in 2.5.3.

For leaf treatments, the spore solution of *Trichoderma* spp. was gained the same way as for greenhouse trials in 2.7.2. In total, 3l of spore solution with a spore density of $1x10^7$ spores/ml were needed for every treatment. All spore solutions and the additional products were applied with a backpack sprayer in a 7-15 day interval. In the years from 2016 to 2018, four applications were carried out each year, and in 2019 the fields were treated five times (Tab. 13). Disease progression was assessed every week, as described in 2.5.3.

Table 13 General parameters for *Trichoderma* field trials 2016-2019.

Year	**2016**	**2017**	**2018**	**2019**
Location	Weihenstephan	Weihenstephan	Weihenstephan	Weihenstephan
Soil	Silt-loam	Silt-loam	Silt-loam	Silt-loam
Cultivar	Lady Amarilla	Maxilla	Lady Amarilla	Maxilla
Plot size	4x4.5m	4x4.5m	4x4.5m	4x4.5m
Planting date	10.05.2016	11.05.2017	11.04.2018	09.04.2019
Emergence	12.06.2016	14.06.2017	11.05.2018	19.05.2019
Isolates for inoculation	*A. solani* 615 (wild type)	*A. solani* 615 (wild type)	*A. solani* 615 (wild type)	*A. solani* 615 (wild type)
Date of inoculation	29.06.2016	21.06.2017	30.05.2018	14.06.2019
Inoculation density	5g infected kernels/m²	5g infected kernels/m²	5g infected kernels/m²	5g infected kernels/m²
Treatments	*T. atroviride* I *T. atroviride* II *T. harzianum* I *T. harzianum* II *T. hamatum* *T. asperellum* TrichoStar®[1] (15l/ha) Tmix Plus®[2] (1kg/ha) Bravo®[4] (2l/ha) control	*T. atroviride* I *T. atroviride* II *T. harzianum* I *T. harzianum* II *T. hamatum* *T. asperellum* Mixture[5] TrichoStar® (15l/ha) Tmix Plus® (1kg/ha) Bravo® (2l/ha) control	*T. atroviride* I *T. atroviride* II *T. harzianum* I *T. harzianum* II *T. hamatum* *T. asperellum* Mixture[5] TrichoStar® (15l/ha) Tmix Plus® (1kg/ha) Bravo® (2l/ha) control	*T. atroviride* I *T. atroviride* II *T. harzianum* II *T. hamatum* *T. asperellum* Bravo® (2l/ha) control
Application dates	19.07.2016 28.07.2016 03.08.2016 13.08.2016	13.07.2017 28.07.2017 14.08.2017 21.08.2017	21.06.2018 03.07.2018 12.07.2018 19.07.2018	03.07.2019 18.07.2019 25.07.2019 01.08.2019 08.08.2019

[1]Intrachem Bio Deutschland GmbH & CO.KG (*T. harzianum* T58)

[2]Intrachem Bio Deutschland GmbH & CO.KG (*Pseudomonas* sp., *Bacillus* sp., *Streptomyces* sp., *T. harzianum* T58, Mycorrhiza)

[4]Syngenta (500g/l Chlorothalonil)

[5]Mixture= Spore solutions of *T. harzianum* I, *T. atroviride* II, *T. hamatum*; 10^7spores/ml each

2.8. Statistics

For statistical analysis, Graph Pad Prism 8 was used. Depending on the homogeneity of variances, an analysis of variances (ANOVA) with a post hoc Tukey test (no significant differences in variance), or a post hoc Tamhane's T2 test (significant differences in variance) was performed. For correlation analysis, the Pearson's r correlation was calculated. To check for significant differences between two samples with unequal variances, I performed a Welch's unequal variance t-test.

3. Results

3.1. Epidemiological studies

To get better knowledge about the epidemiology of *A. solani*, I analyzed the following aspects in more detail: latent period, influence of leaf moisture, the impact of temperature and the spectrum of non-host or alternative host plants.

3.1.1. Latent period

To test the latent period, 10 randomly picked *A. solani* isolates were used for spray inoculation in greenhouse. All strains lead to first tiny symptoms one day after inoculation (Fig. 1).

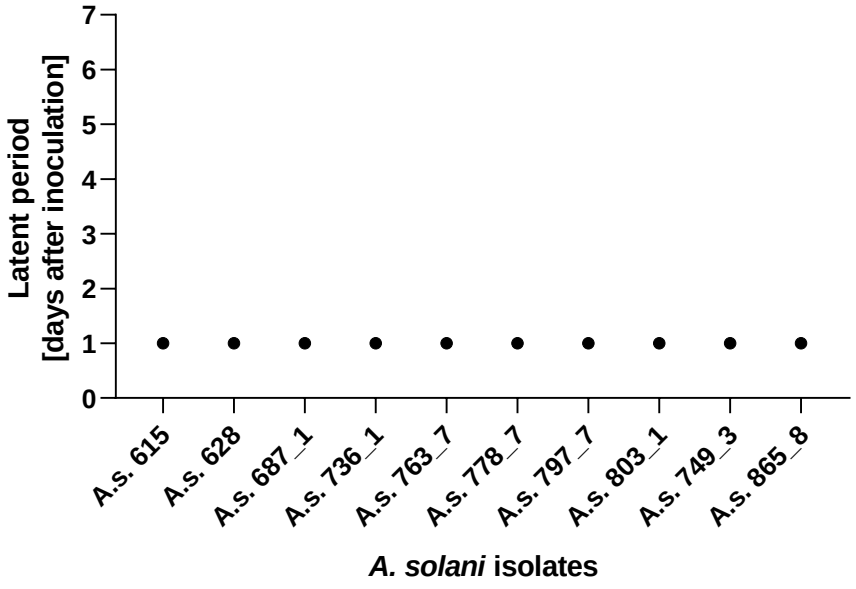

Figure 1 Latent period of different *A. solani* isolates in greenhouse trials. For each isolate, three replicate plants were infected. Spore density was adjusted to $1x10^4$ spores/ml for each strain. The whole setup was repeated three times with similar results.

3.1.2. Leaf moisture

Leaf moisture is a key factor for successful infection of potato by *A. solani* and future climatic changes may have an impact on leaf moisture duration on leaves in general. Based on that, I wanted to assess the minimum leaf moisture duration period that is needed for successful infection with *A. solani* on potato. Therefore, I performed a greenhouse trials to assess the influence of different periods of leaf moisture on successful infection with *A. solani*. The infected plants were removed from the infection tent with 100% relative humidity 6, 8, 10, 12, 16, 18, 20, 22, and 24h after inoculation. For each time point, three *A. solani* isolates with three replicate plants per isolate were used for this trial. The infected leaf area was rated seven days after infection for all variants. The whole setup was independently repeated three times.

Overall, I observed increasing infected leaf areas with longer periods of leaf moisture (Fig. 2). Because all three tested *A. solani* isolates behaved similarly, they were summed up for the analysis. According to Tamhane's T3 test, the infection with 8, 10, and 12h of leaf moisture (2.2; 3.8; 3.4% respectively) was significantly higher compared to the 6-hour variant (0.2%). The disease

development for the variants with 16 and 18h was again significantly higher compared to the shorter periods (6.3 and 7.7%, respectively). There was also a significant difference between 20 (10%) and 16h. The two most extended periods of leaf moisture (22 and 24h), again led to significantly more infected leaf areas compared to all other variants (13.7 and 17.7%, respectively).

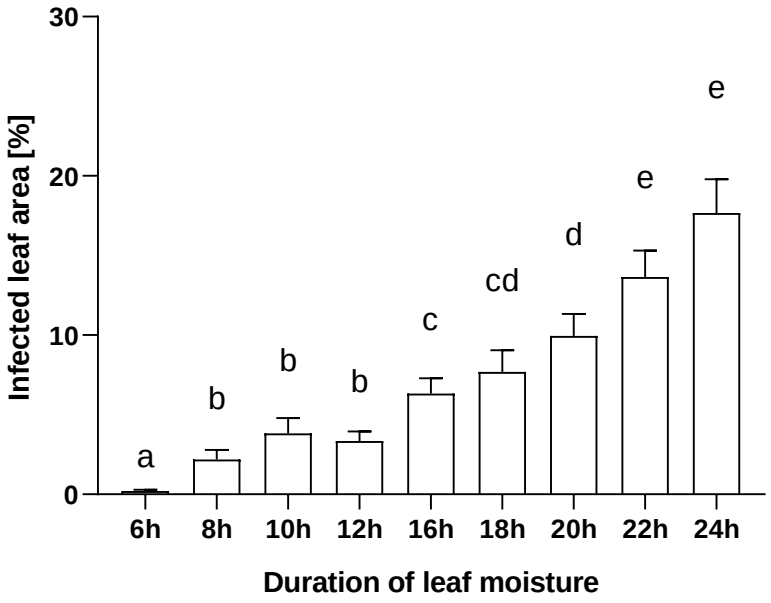

Figure 2 Influence of leaf moisture duration on infection with *A. solani* in greenhouse 7dai. For each time point, three different *A. solani* isolates with three replicate plants per isolate were used. Spore density was adjusted to $1x10^4$ spores/ml for each strain. The whole setup was repeated three times with similar results. Error bars indicate 95% CI. Bars with no significant differences are marked with the same letter, according to Tamhane's T2 test ($p<0.05$) post hoc test after ANOVA (n=27).

3.1.3. Temperature

Based on predicted climatic changes, e.g. rising temperatures, it is very important to get more profound knowledge about the consequences of these changes on pathogens and their caused diseases. Therefore, two independent greenhouse trials were carried out to assess the influence of different temperatures on the disease development. During the first 30h after inoculation with two *A. solani* strains, the conditions were the same for all variants. Afterwards, the plants were incubated in climate chambers with either 18, 22, or 26°C for another six days before symptoms were rated.

The results from the first repetition showed a clear trend: for both isolates, the 26°C lead to a higher infection rate after seven days than the lower temperatures (Fig. 3). The *A. solani* isolate 808_4 also showed by trend more infection at 22°C compared to 18°C. This difference was not visible for the infection with *A. solani* 754_2 in the first repetition.

In the second repetition, no clear difference was observed between disease developments under different temperatures for *A. solani* 808_4 (Fig. 3). The infection success of *A. solani* 754_2, however, appeared to be supported at higher temperatures – similar to the first repetition.

Overall, higher temperatures of 26°C appear to increase infection success of both tested *A. solani* isolates.

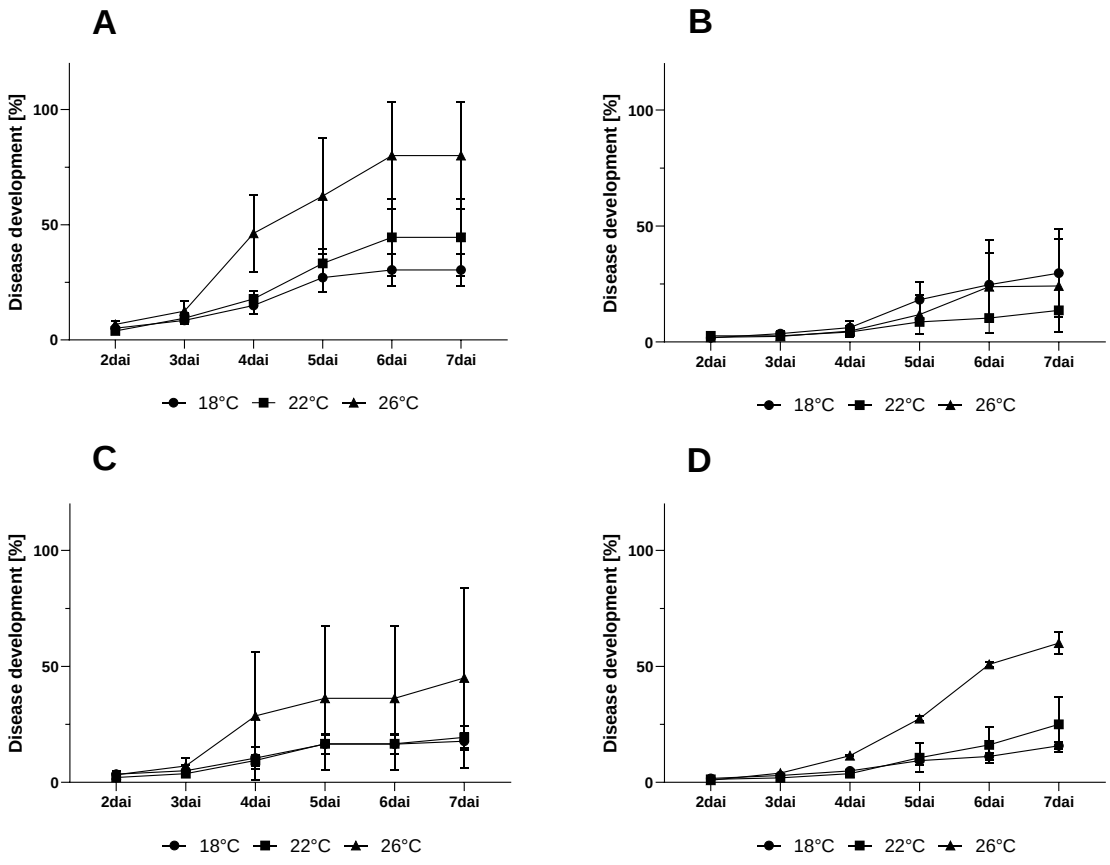

Figure 3 Development of *A. solani* infection dependent on different temperatures during the progression phase. Infection conditions during the first 24h were the same for all variants. Disease development of *A. solani* 808_4: first repetition (A) and second repetition (B). Disease development of *A. solani* 754_2: first repetition (C) and second repetition (D). Each data point shows the mean out of two (in one case one) to four replicate plants. Error bars indicate SD.

3.1.4. Weather data analysis

Based on my findings about the minimum leaf moisture duration for successful infection with *A. solani* (8h) and the predicted climatic changes (e.g. rising temperatures, drought periods), I wanted to assess whether these changes affect leaf wetness or not. Therefore, I analyzed weather data from 01.05. to 31.08 for 2016 to 2019. In a first step, I correlated the parameters temperature and precipitation – which are predicted to change in future – with leaf moisture. In addition to that, I also analyzed the correlation between RH and leaf moisture. In a last step, I assessed the impact of precipitation on RH. For all parameters, the calculations are based on hourly values. Weather data for these analyses derived from the weather station Dienstweiler in Rhineland Palatinate, because there the leaf wetness is directly measured and not calculated like in Freising. In a second approach, I wanted to answer the question, if we still can find this minimum leaf wetness period of 8h in the field. To answer this, I analyzed weather data from the weather station Freising and used the parameter RH as indicator for leaf moisture.

For analysis of the dependency of leaf moisture on precipitation, a linear regression with a Pearson's r correlation was performed with the hourly values from 01.05 to 31.08 for each year (Fig. 4). For all

years, a very low significant positive correlation was found. R^2 values varied between 0.02683 and 0.06050 with P values under 0.0001.

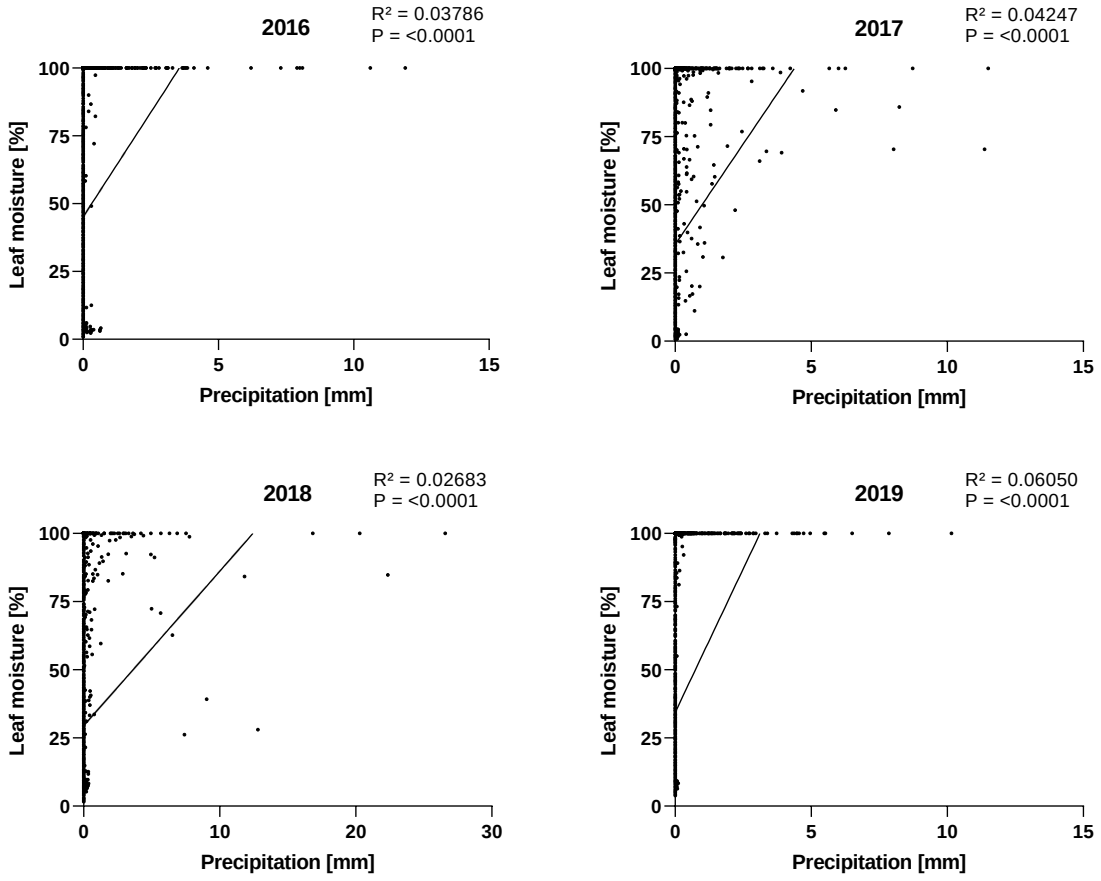

Figure 4 Linear regression and Pearson r correlation between precipitation [mm] and leaf moisture [%] for the years 2016 to 2019. Hourly values from 01.05. until 31.08. are displayed for each year.

When we now compare the relation between temperature and leaf moisture, significant positive correlations can be found in all years – R^2s were calculated between 0.1846 and 0.3039 with a P value below 0.0001 (Fig. 5).

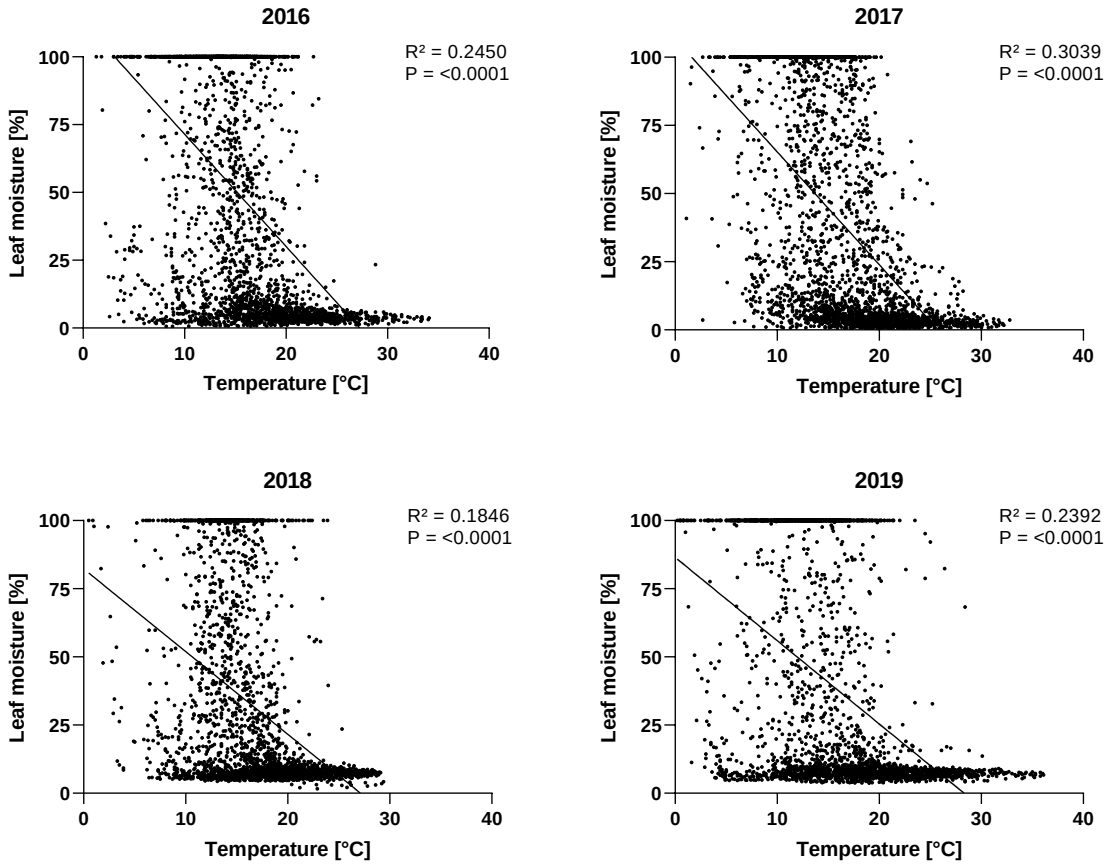

Figure 5 Linear regression and Pearson r correlation between temperature [°C] and leaf moisture [%] for the years 2016 to 2019. Hourly values from 01.05. until 31.08. are displayed for each year.

In the next step, I checked the correlation between relative humidity and leaf moisture with a Pearson's r correlation (Fig. 6). For all four years (2016 to 2019), a positive correlation could be observed between relative humidity and leaf moisture (R^2 of 0.6303, 0.5761, 0.4124, and 0.6276 for the respective years). All P values were <0.0001.

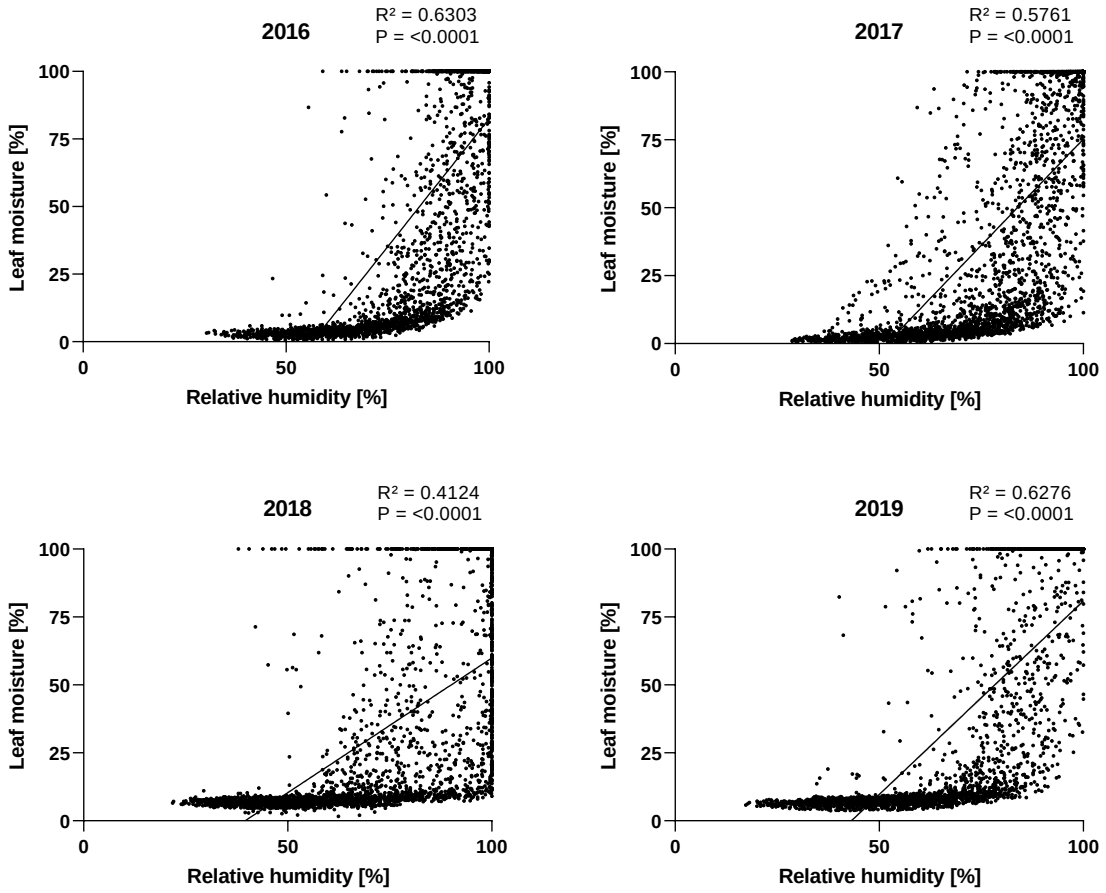

Figure 6 Linear regression and Pearson r correlation between relative humidity [%] and leaf moisture [%] for the years 2016 to 2019. Hourly values from 01.05. until 31.08. are displayed for each year.

Last, I analyzed the correlation between precipitation and relative humidity for all four years. A positive correlation with very low R^2 values was observed in all years (R^2 from 0.01763, 0.02109, 0.01241 and 0.03195, for each year, respectively (Fig. 7). The P values for all correlations were <0.0001.

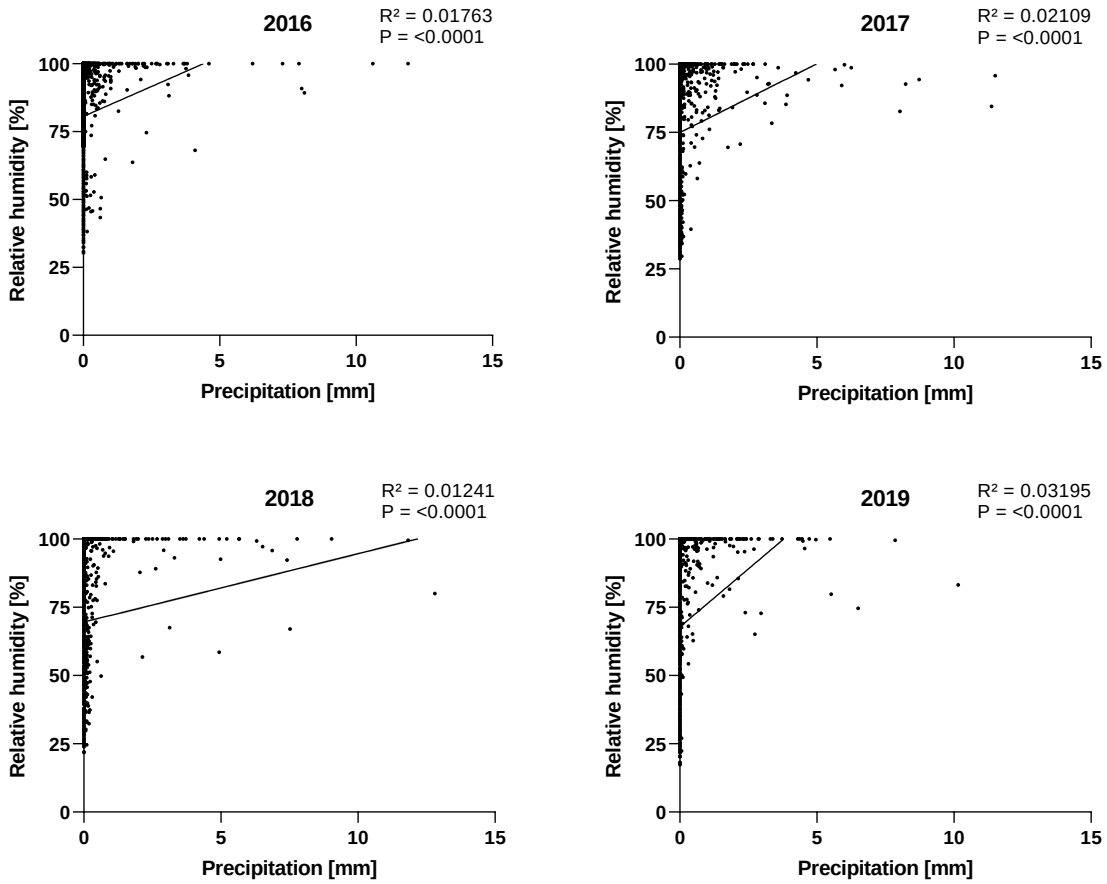

Figure 7 Linear regression and Pearson r correlation between precipitation [mm] and relative humidity [%] for the years 2016 to 2019. Hourly values from 01.05. until 31.08. are displayed for each year

To get an overview, how many days per month would potentially be favorable for *Alternaria* infection in the field, not only RH was taken into account, but also temperature. With this additional parameter, I wanted to exclude periods with potential enough leaf wetness, but too low temperatures for the fungus to infect plants at all. For the analysis defined combinations of relative humidity and temperature were used.

In several models, 90% relative humidity is taken as an indicator for leaf wetness (Wilks and Shen 1991; Sentelhas et al. 2008). Therefore, I decided to use a minimum of 90% relative humidity and, in another scenario, at least 95% as parameter for leaf wetness in the field, to minimize the amount of overestimation. Only those days with a humidity of at least 90 or 95% for a minimum of 8 hours in a row were counted.

In addition to the specifications for the RH, the minimum required temperature during these specified 8 hours was considered to be at least 10°C. In a second scenario, the minimum temperature was set to 15°C. In total, we thus screened our weather data for four different combinations:

≥90% RH and ≥10°C; ≥90% RH and ≥15°C; ≥95% RH and ≥10°C; ≥95% RH and ≥15°C

With these defined requirements, the four months – May, June, July, and August – were analyzed for each year. Overall, every month and year, critical days were detectable (Tab. 16). The mean number of days with at least 90% RH and 10°C was 8 in May, 13 in June, 16 in July, and 19 in August. Out of these days, a mean of 0.5, 5, 4, and 5 days respectively recorded a minimum temperature of 15°C. For the specifications with at least 95% RH and 10°C, a mean number of 5, 10, 12, and 15 days respectively, was calculated. Out of these days, a mean of 0.025, 3, 3, and 4 days respectively, were recorded with at least 15°C during the 8 hours of leaf wetness.

Table 14 Total number of days per month with consecutively at least 8h of either 90 or 95% relative humidity (RH) and a minimum temperature of 10°C. Numbers in brackets indicate days with a minimum temperature of 15°C.

	Days with min. 8h >90% RH min. 10°C				**Days with min. 8h >95% RH min. 10°C**			
Year	**May**	**June**	**July**	**Aug**	**May**	**June**	**July**	**Aug**
2016	6 (0)	13 (1)	15 (6)	12 (0)	1 (0)	4 (0)	10 (4)	12 (0)
2017	4 (1)	8 (4)	12 (3)	21 (7)	3 (1)	7 (2)	10 (3)	15 (5)
2018	13 (1)	16 (6)	20 (4)	22 (6)	9 (0)	14 (6)	19 (4)	18 (6)
2019	8 (0)	16 (7)	15 (3)	20 (6)	6 (0)	15 (4)	8 (1)	16 (3)
Mean	8 (0.5)	13 (5)	16 (4)	19 (5)	5 (0.25)	10 (3)	12 (3)	15 (4)

3.1.1. *A. solani* from alternative host plants

In the context of possible inoculum sources of *A. solani* in agricultural fields, I wanted to evaluate the pathogenicity of *A. solani* strains from alternative hosts on the cultivated potatoes. For these experiments different fungal isolates were tested: three potato leaf, potato tuber, tomato, and thistle isolates, two wild tomato isolates, and one black nightshade isolate. Besides the general potential for infection of potato plants, latent period, spore density, and spore germination were taken into account.

All tested isolates were able to infect potato plants in greenhouse trials (Fig. 8). The variance between the infected leaf area after seven days for the three independent experiments is high for some hosts (tomato, black nightshade, and thistle). Therefore, no clear pattern is visible regarding the most aggressive isolates/original hosts.

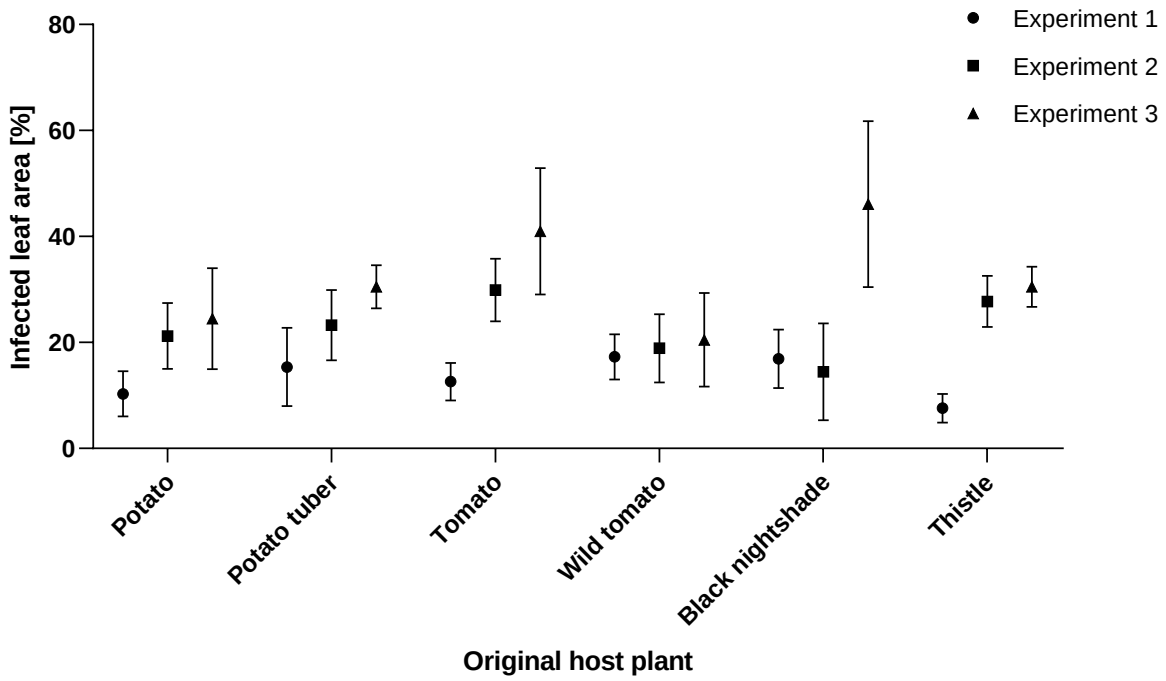

Figure 8 Infected potato leaf area of *A. solani* isolates from potato and alternative host plants [%] seven days after inoculation in greenhouse trials. In total, three potato, potato tuber, tomato, and thistle isolates, two wild tomato isolates, and one black nightshade isolate were tested. For each strain, three replicate plants were infected. Spore density was adjusted to $1x10^4$ spores/ml for each isolate. The whole experiment was repeated three times with similar results. Error bars indicate 95% CI.

In addition to the assessment of disease development seven days after inoculation, the latent period was also determined. For all tested isolates from diverse original hosts, first symptoms were visible one day after inoculation (Fig. 9).

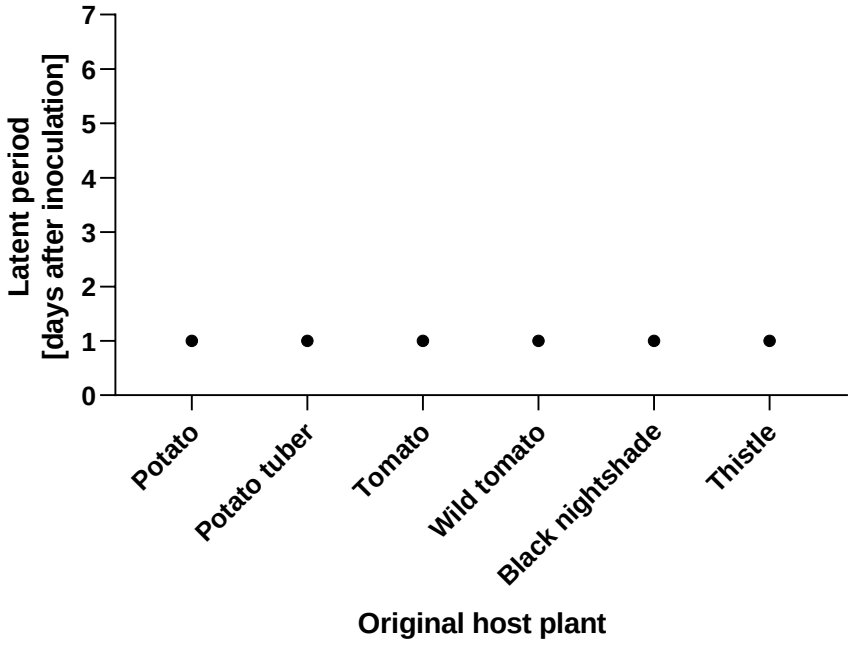

Figure 9 Latent period of *A. solani* isolates from different hosts in greenhouse trials. In total, three potato, potato tuber, tomato, and thistle isolates; two wild tomato isolates and one black nightshade isolate were tested. For each strain, three replicate plants were infected. Spore density was adjusted to $1x10^4$ spores/ml for each isolate. The whole setup was repeated three times with similar results.

Besides the greenhouse trials, I conducted an *in vitro* experiment to assess the spore production potential and the spore germination rate of the respective isolates used in the greenhouse trials.

Regarding the isolates from the hosts potato, potato tuber, and tomato, no clear pattern is visible for either spore density or spore germination. Some of these isolates show much higher spore densities or germination rates than other strains from the same host. In contrast, the isolates from thistle and wild tomato behaved quite similarly. They showed low spore densities and high spore germination rates (Fig. 10).

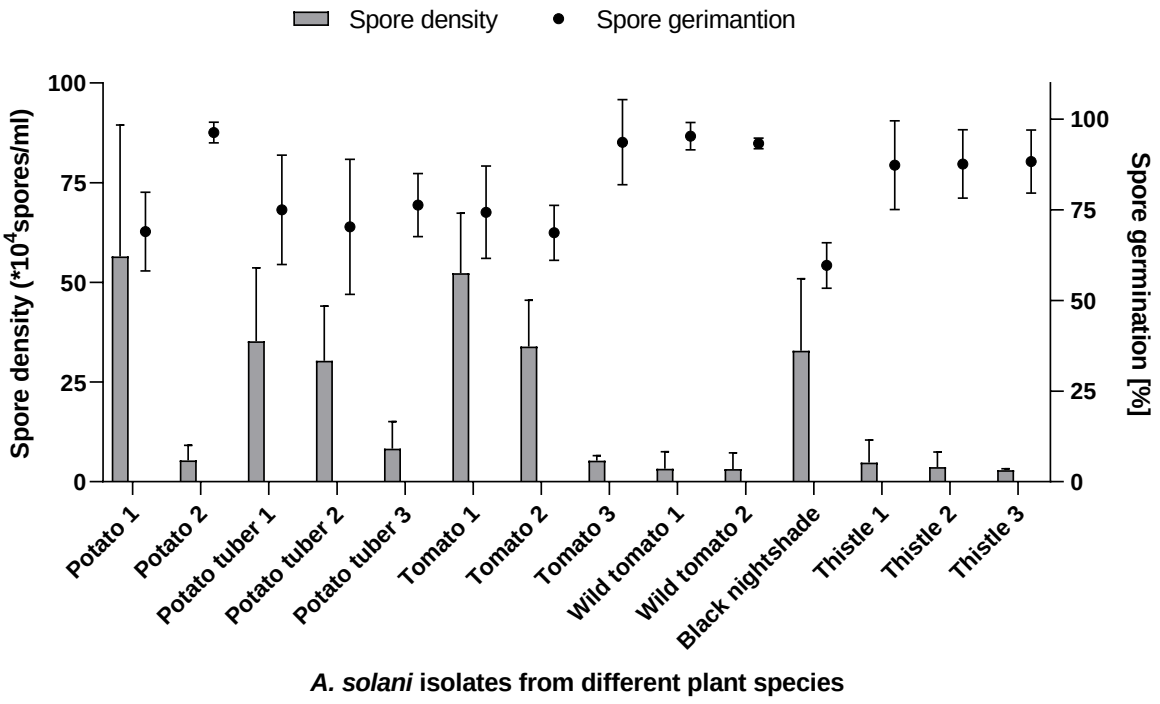

Figure 10 Spore density and spore germination of *A. solani* isolates from different potential hosts. For each isolate and parameter, three replicate plates were measured. Error bars indicate 95% CI.

Regardless of the different original hosts, there is a negative correlation visible between the density or formed spores and the germination rate of spores according to Pearson's r correlation test: R^2 = 0.6385 and P = 0.0006 (Fig. 11).

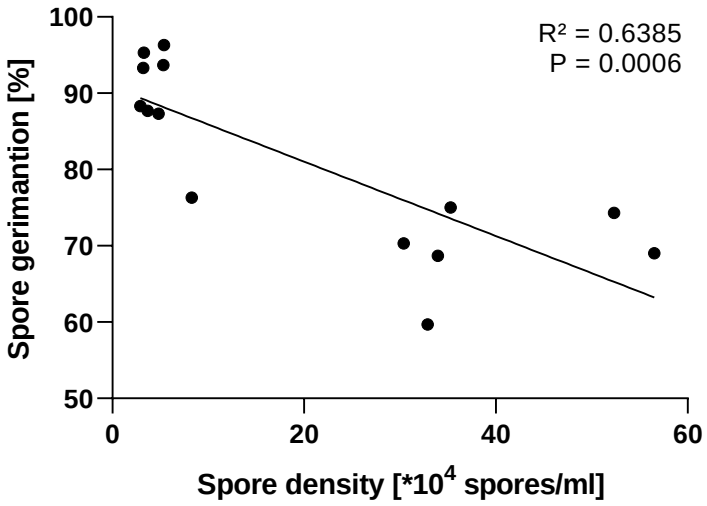

Figure 11 Linear regression and Pearson r correlation between spore germination and spore density of *A. solani* isolates from diverse original hosts. Each dot represents one isolate. In total, 14 isolates were tested.

3.2. Development of resistance against SDHIs

The occurrence of mutations, which negatively influence fungicide sensitivity, is a severe problem in chemical plant protection. Therefore, it is very important to have an overview of the occurrence and spreading of particular mutations and in the next step to estimate the consequences of these

mutations on fungicide sensitivity. In a first step, the prevalence of *Sdh*-mutated isolates was assessed for several locations all over Germany between 2013 and 2016. Afterwards, the sensitivity of some isolates against boscalid – one prominent active fungicide ingredient from the SDHI group – was evaluated *in vitro*, *in vivo,* and in the field. In addition, a subset of the isolates was also tested for their sensitivity against the SDHI fluopyram *in vitro* to analyze possible crossresistance. Beside Cantus®, with its active ingredient boscalid, some other SDHI-fungicides with different active ingredients were also included in the field trials to get a broader picture of the efficacy of several SDHIs.

3.2.1. Monitoring 2013-2016 in Germany

Between 2013 and 2016, leaf samples with typical early blight symptoms from several locations in Germany (Fig. 12) were used to isolate *A. solani* strains and sequence their *Sdh*-gene regions for possible mutations. In total, 22, 28, 34, and 26 locations were analyzed from 2013 to 2016, respectively (Tab. 14). The number of sites with *Sdh*-wild type isolates decreased over time, whereas especially the presence of *SdhB*-H278Y and *SdhC*-H134R mutant strains constantly increased (Tab. 14). The previously undescribed *SdhC*-H134Q mutation was only found in a limited amount of isolates in 2014 and 2015. *SdhD*-D123E mutations were only found in 2015 in low numbers.

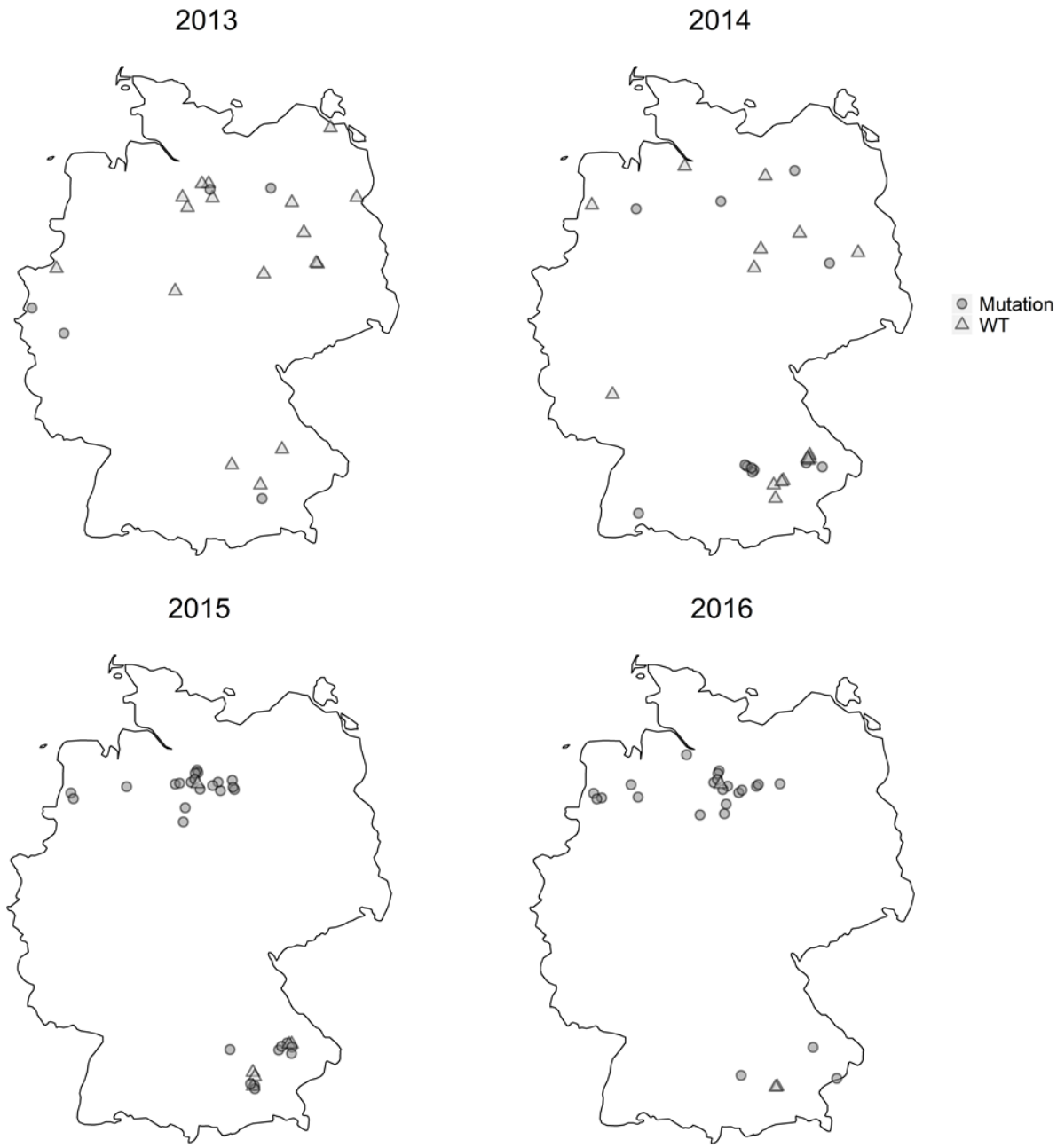

Figure 12 Locations with and without *Sdh*-mutated genotypes for the years 2013-2016. (Δ) Location without *Sdh*-mutated isolates. (○) Site with at least one *Sdh*-mutated strain. For each location, nine to thirteen isolates were analyzed.

Table 15 Number of locations with the respective genotype [%] from 2013-2016 (table from Metz et al. (2019))

			Number of isolates with the respective genotypes						
Year	Number of locations	Number of isolates	WT (%)	*SdhB*-H278R (%)	*SdhB*-H278Y (%)	*SdhC*-H134R (%)	*SdhC*-H134Q (%)	*SdhD*-D123E (%)	*SdhD*-H133R (%)
2013	22	137	100	0	5	9	0	0	0
2014	28	67	93	0	0	7	4	0	0
2015	34	196	44	3	18	62	3	3	0
2016	26	325	35	4	35	69	0	0	0

3.2.2. Boscalid sensitivity *in vitro*

For assessing the boscalid sensitivity of different *A. solani* isolates, I determined the EC_{50} values *in vitro* using the commercial product Cantus® with boscalid as active ingredient. In total, 47 *A. solani* isolates – with and without *Sdh*-mutations – were tested for their sensitivity to boscalid, including 20 *Sdh*-wild type, 11 *SdhB*-H278Y, 2 *SdhC*-H134Q, and 14 *SdhC*-H134R isolates (Tab. 15). All tested *Sdh*-wild type isolates showed a sensitive response to boscalid treatments. Out of the 11 *SdhB*-H278Y strains, one isolate was categorized as moderately resistant and the 10 others as highly resistant. Regarding the *SdhC*-H134R mutation, one isolate out of 14 responded sensitive to boscalid, one moderately and 12 highly resistant.

Table 16 Sensitivity of *A. solani* isolates to boscalid according to their EC_{50} values *in vitro*.

***Sdh*-genotypes**	**Total number of strains**	**Number of sensitive strains (EC_{50} <5µg/ml)**	**Number of moderately resistant strains (EC_{50} 5 to 20µg/ml)**	**Number of highly resistant strains (EC_{50} >20µg/ml)**
***SdhB*-H278Y**	11	0	1	10
***SdhC*-H134Q**	2	1	1	0
***SdhC*-H134R**	14	1	1	12
***Sdh*-wild type**	20	20	0	0

3.2.3. Boscalid sensitivity *in vivo*

In the next step, I tested the sensitivity of 17 *A. solani* isolates against boscalid on plants in the greenhouse, to evaluate the impact of *Sdh*-mutations on fungicide resistance under more natural conditions. In total, the fungicide efficacy of 100µg boscalid was evaluated for three *Sdh*-wild type, two *SdhB*-H278R, four *SdhB*-H278Y, two *SdhC*-H134Q, and six *SdhC*-H134R isolates.

Overall, the controlling effect of boscalid against *Sdh*-mutants was significantly lower compared to the *Sdh*-wild type inoculation (99%), when plants were infected with *SdhB*-H278R (75%), *SdhB*-H278Y (43%) or *SdhC*-H134R mutant strains (47%) (Fig. 13). Only the efficacy for the *SdhC*-H134Q genotypes (85%) was not significantly different to the *Sdh*-wild type inoculation (Fig. 9). Moreover, the control of the *SdhB*-H278Y and *SdhC*-H134R mutant strains was significantly less effective compared to the *SdhB*-H278R strains.

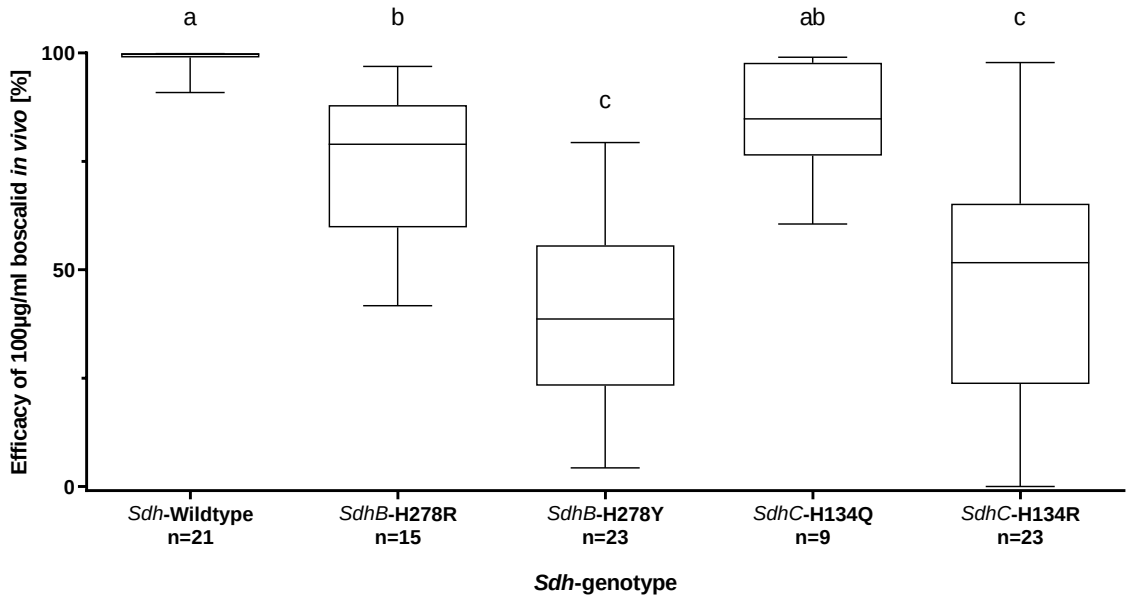

Figure 13 Mean fungicide efficacy of different *Sdh*-regions tested with 100µg/ml boscalid In greenhouse trials. For *Sdh*-wild type, the number of tested isolates is n=3, for *SdhB*-H278R n=2, for *SdhB*-H278Y n=4, for *SdhC*-H134Q n=2 and for *SdhC*-H134R n=6. Spore density was adjusted to $1x10^4$ spores/ml for each isolate. For each strain, at least two independent greenhouse trials with three plants per treatment were conducted. Whiskers indicate Min to Max. Boxes marked with the same letters are not significantly different according to post hoc Tamhane's T2 test (a=0.05) post hoc test after ANOVA.

3.2.4. Boscalid sensitivity in the field

Meteorological data

In 2016, the temperatures in the planting period from April to May varied between 8 and 13°C with an average rainfall of 6mm per week (except for three weeks of more torrential rainfalls mid of April, mid and end of May) (Fig. 14). During the potato growing period in June, mean temperatures increased to 16°C with precipitations about 22mm each week. In the period of early blight progression from July to August, the temperatures rose to 18°C, and a mean rainfall of 23mm each week was measured until mid of August. The last weeks until the end of September were dry except for one week, and mean temperatures between 11 and 18°C were measured. The average leaf moisture during the disease progression was recorded with 38%.

The mean temperatures during the planting period in 2017 were comparable to 2016 (Fig. 14). While this period, there were four weeks of heavy rainfall between mid-April till mid-May – on average 35mm per week. Within the potato growing phase in June, average rainfalls of 4-15mm per week and temperatures around 19°C were recorded. In July and August, constant rainfalls with an average of 31mm were recorded for most of the weeks. From June to August, a mean temperature of 19°C and an average leaf moisture of 40% was measured. Regarding the leaf moisture, there were six weeks between June and July, where an average of 30% per week was recorded, while the temperature was still around 19°C. In September, the temperature and precipitation decreased again.

In 2018, the planting period was arid from April until the beginning of May, with mean temperatures rising from 5 to 14°C (Fig. 14). In May and June, an average precipitation of 27mm, including two weeks with 60 and 77mm and two weeks with under 6mm, was recorded. For the rest of the season,

a mean rainfall of 16mm per week were measured except for two consecutive weeks with nearly no rain end of July. The mean temperatures from mid of May until the end of the season were around 19°C, with a peak of 23°C in the last week of July and the first week of August. The average leaf moisture from May to August was around 48%.

In 2019, the weather conditions of the planting period were characterized by a low amount of precipitation in April and May with around 7mm per week (except for one week in May with 73mm) and fluctuating mean temperatures from 9 to 14°C (Fig. 14). Especially during the first three weeks of April, nearly no rain was recorded. During disease progression from mid of June until the end of August, a mean precipitation of 18mm per week, with two weeks of heavy rain (50mm each), and a mean temperature of 19°C was recorded. The average leaf moisture between June and August was 45%.

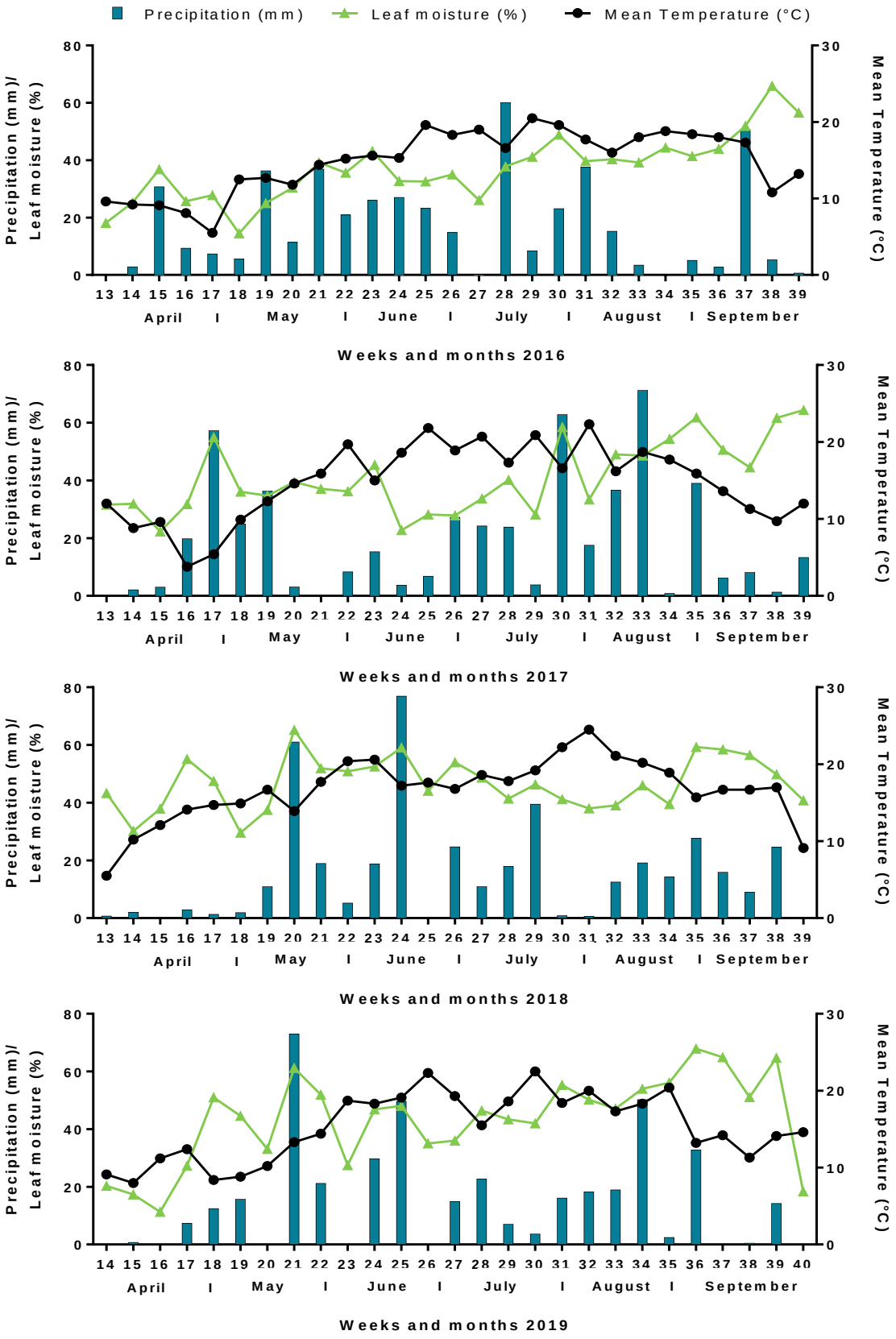

Figure 14 Weekly mean of meteorological conditions from the first week of April until the last week of September in the years 2016-2019 in Freising. Data source Bavarian State Institute for Agriculture – weather station "Freising".

Disease assessment

Between 2016 and 2018, I conducted field trials to assess the influence of *Sdh*-wild type and two different *SdhC*-H134R mutant inoculations on the efficacy of the commercial product Cantus® with its active ingredient boscalid.

For the years 2016 and 2017, the general disease level of the natural early blight infection was similar with calculated rAUDPC values of around 0.3 (Fig. 15). In 2018, the rAUDPC of the naturally infected plots was about 0.55. For comparison of the rAUDPC values of the untreated control and the Cantus® treatment within each of the three inoculations, a t-test was performed for each year. In 2016, the Cantus® treatment in the *Sdh*-wild type and *SdhC*-H134R mutant 1 inoculation (0.22 and 0.21, respectively) lead to a significant reduction of the rAUDPC compared to their untreated controls (0.38 and 0.33 respectively). In the *SdhC*-H134R mutant 2 inoculation, no difference was observed between Cantus® treated and non-treated plots (0.47 and 0.48, respectively). In 2017, a significant difference was observed between the Cantus® treatment (0.21) and the untreated control (0.38) after *Sdh*-wild type inoculation. In both *SdhC*-H134R mutant inoculations, no significant difference was detected between the fungicide treated and non-treated controls. Similar results were observed in 2018. The disease level was significantly reduced after Cantus® treatments (0.39) compared to the untreated control after *Sdh*-wild type inoculation (0.52). After *SdhC*-H134R mutant inoculations, no significant reduction of infected leaf area in the Cantus® treated plots was assessed, and the rAUDPC was for all variants around 0.6.

For evaluation of the success of the kernel infection, the differences between the rAUDPC values of the fungicide-untreated controls in the naturally infected plots and the untreated plots in the artificially inoculated plots were statistically analyzed.

In 2016, the rAUDPC values of the untreated controls after *Sdh*-wild type and *SdhC*-H134R mutant inoculations were significantly higher than the untreated control after natural infection. Regarding the untreated control of the *SdhB*-H278Y inoculation, only a by trend higher rAUDPC could be observed compared to the natural disease development.

In 2017, all three artificially inoculated fungicide-untreated controls showed significantly higher rAUDPC values compared to the natural infection.

In 2018, no significant differences were observed between all untreated controls. The rAUDPC values of the *SdhB*-H278Y and *SdhC*-H134R mutant inoculation showed by trend higher values compared to the natural infection.

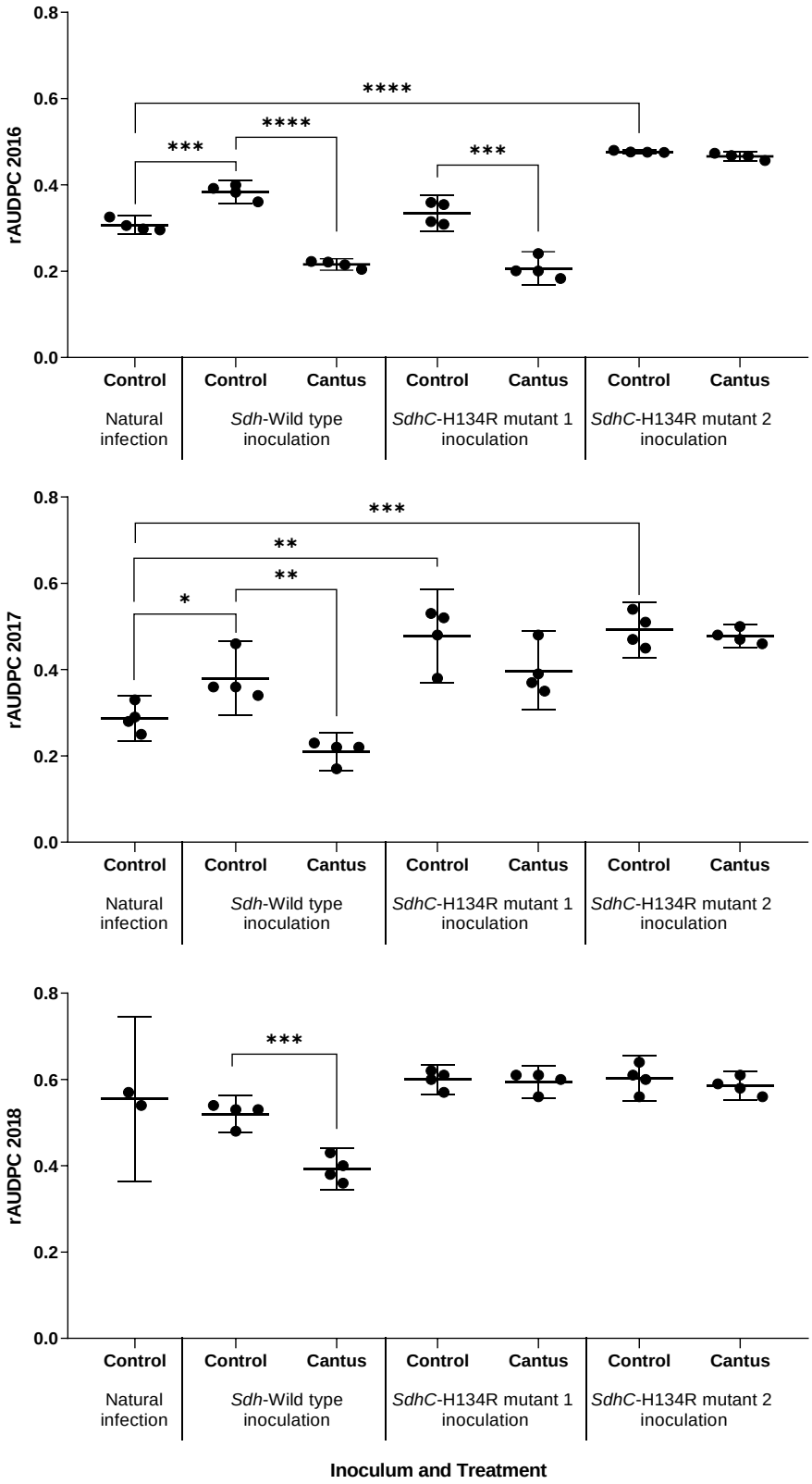

Figure 15 rAUDPC values of different inoculations with their untreated controls compared to fungicide (Cantus®) treatment from field trials 2016 to 2018 with the cultivar Lady Amarilla. Four replicate plots per treatment are shown. Significant differences within each inoculation and between the control in the natural infection compared to the controls of the artificially inoculated plots are marked with a star, according to Tamhane's T2 test ($p<0.05$) post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001.* Error bars indicate 95% CI.

3.2.5. Sensitivity of *A. solani* isolates against two different SDHIs *in vitro*

For a sensitivity testing against boscalid and fluopyram *in vitro* I picked randomly twenty *A. solani* isolates. In total, the EC_{50} value of 6 *Sdh*-wild type, 5 *SdhB*-H278Y, 4 *SdhC*-H134Q, and 5 *SdhC*-H134R mutant isolates was calculated. For the boscalid sensitivity, I classified the EC_{50} values into four groups. Group definitions were made on basis of Gudmestad et al. (2013), but adjusted to our results: EC_{50}<5; <20; <100; >100µg/ml. All isolates were much more sensitive towards fluopyram, so they were classified into the following four groups: EC_{50}<0.5; <1; <5; >5µg/ml.

Regarding the boscalid sensitivity, all six *Sdh*-wild type isolates showed EC_{50} values below 5µg/ml (Tab. 17). For the *SdhB*-H278Y mutants, two had EC_{50} values under 20µg/ml and three over 100µg/ml. Out of four isolates with *SdhC*-H134Q mutation, three showed EC_{50} values under 20µg/ml and one under 100µg/ml. The EC_{50} values of the five *SdhC*-H134R mutants varied more: one was under 5µg/ml, one under 20µg/ml, one under 100µg/ml and two over 100µg/ml boscalid.

The calculated EC_{50} values for fluopyram were all under 5µg/ml (Tab. 17). In detail, four out of six *Sdh*-wild type isolates were classified under 0.5µg/ml, one under 1µg/ml and one under 5µg/ml. From the five *SdhB*-H278Y isolates, four showed EC_{50} values under 0.5µg/ml and one under 1µg/ml. All four tested *SdhC*-H134Q strains had EC_{50} values under 5µg/ml. Three of the *SdhC*-H134R mutant isolates showed EC_{50} values under 1µg/ml, and two isolates had values under 5µg/ml fluopyram.

Table 17 Total number of isolates, which belong to defined groups according to their sensitivity to the active ingredient boscalid and fluopyram *in vitro*. In total, the EC_{50}value of 6 *Sdh*-wild type, 5 *SdhB*-H278Y, 5 *SdhC*-H134R, and 4 *SdhC*-H134Q isolates was determined. For boscalid, the values were put into four different classes (<5; <20; <100; >100µg/ml) and for fluopyram four classes were defined (<0.5; <1; <5; >5µg/ml).

***Sdh*-genotype**	**EC_{50}values for boscalid (µg/ml)**				**EC_{50}values for fluopyram (µg/ml)**			
	<5.0	<20.0	<100.0	>100.0	<0.5	<1.0	<5.0	>5.0
***Sdh*-wild type**	6	0	0	0	4	1	1	0
***SdhB*-H278Y**	0	2	0	3	4	1	0	0
***SdhC*-H134Q**	0	3	1	0	0	0	4	0
***SdhC*-H134R**	1	1	1	2	0	3	2	0

To compare the sensitivity of single *A. solani* isolates towards boscalid and fluopyram, the EC_{50} values of 6 *Sdh*-wild type, 5 *SdhB*-H278Y, 4 *SdhC*-H134Q and 5 *SdhC*-H134R mutant isolates were plotted together for both active ingredients (Fig. 16). Because of the high range within the EC_{50} values of boscalid, they are shown on a logarithmic scale from 0.00001 to 100000.0. The scale for EC_{50} values of fluopyram reaches from 0.0 to 4.0.

The EC_{50} values of the *Sdh*-wild type isolates for both active ingredients are rather low.

The fluopyram sensitivity of the tested isolates with a *SdhB*-H278Y mutation was low (EC_{50} <1.0). For the sensitivity against boscalid, a wide range was observed (EC_{50} <10.0 and up to <10000.0) (Fig. 16).

For the *SdhC*-H134Q group, the four isolates showed EC_{50} values between 2.0 and 3.2 for fluopyram and between 6.0 and 44.0 for boscalid (Fig 16).

Within the five *SdhC*-H134R mutant isolates, a slight tendency is visible: The two strains with the highest EC_{50} values for boscalid also show the highest EC_{50} values for fluopyram. The three remaining isolates show comparable values for fluopyram (around 1.0), but different values for boscalid (between 0.3 and 84.0) (Fig 16).

To check possible correlations between the sensitivity of both active ingredients, a Pearson´s r correlation was performed for all 20 isolates. A genotype-specific correlation is not reasonable because of the low number of tested strains per genotype. No clear correlation could be detected between fluopyram and boscalid sensitivity over all isolates (R^2=0.1805; P=0.0618).

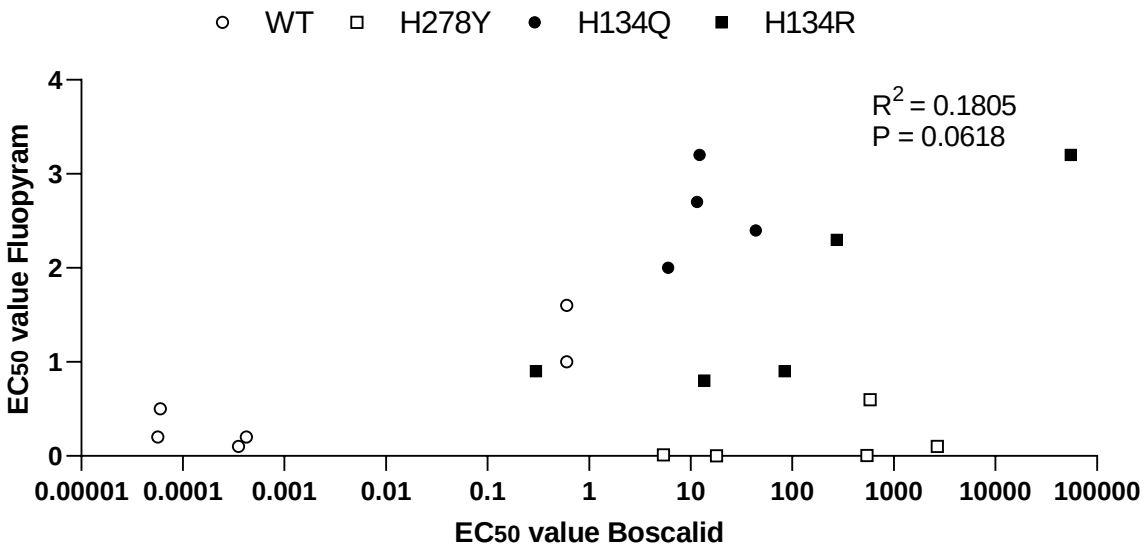

Figure 16 Comparison between EC_{50} values for pure boscalid and fluopyram of single *A. solani* isolates with different *Sdh*-regions: *Sdh*-WT, *SdhB*-H278Y, *SdhC*-H134Q, and *SdhC*-H134R. EC_{50} values of boscalid are shown on the x-axis on a logarithmic scale. Pearson´s r correlation test was performed over all 20 isolates.

3.2.6. Efficiency of different SDHIs in the field

To analyze the efficiency of SDHI-fungicides with different active ingredients under field conditions with artificial inoculation of specific *Sdh*-mutated and *Sdh*-wild type *A. solani* isolates, I conducted field trials between 2016 and 2018. In total, three different SDHI fungicides and untreated controls were included in this setup.

In 2016, all SDHIs led to a significant reduction of the rAUDPC values in the *Sdh*-wild type inoculation (Fig. 17). After *SdhC*-H134R mutant 1 and mutant 2 inoculations, only the SDHI2 and 3 were able to reduce the disease progression compared to their untreated controls significantly.

In 2017, the performance of the SDHIs was, in general, comparable to 2016, but with higher variance within each treatment (Fig. 17). After *Sdh*-wild type and *SdhC*-H134R mutant 2 inoculations, the SDHIs 2 and 3 significantly decreased the rAUDPC values compared to their untreated controls. After *SdhC*-H134R mutant 1 inoculation lead only the SDHI 1 treatment to significantly less disease progression.

For the third year of this trial in 2018, the results were similar to 2016. After *Sdh*-wild type inoculation, all treatments significantly reduced the rAUDPC values compared to the untreated control (Fig. 17). After the two *SdhC*-H134R mutant inoculations, only the SDHI2 and 3 lead to significantly fewer disease symptoms compared to their untreated controls. The SDHI4 showed no significant difference to the control in both mutant inoculations.

During the field trials, there were also some differences in the performance of the single SDHIs visible. Over all three years in the *Sdh*-wild type inoculation, the SDHI2 led to significantly lower rAUDPC values than the other SDHI treatments, and the SDHI3 showed significantly fewer disease symptoms then the SDHI4. After *SdhC*-H134R mutant 1 inoculation, the rAUDPC values of SDHI2 and 3 were not significantly different in all three years. For the *SdhC*-H134R mutant 2 inoculation, significantly lower rAUDPC values were observed for the SDHI2 compared to the SDHI3 treatment in the years 2016 and 2018, but not in 2017.

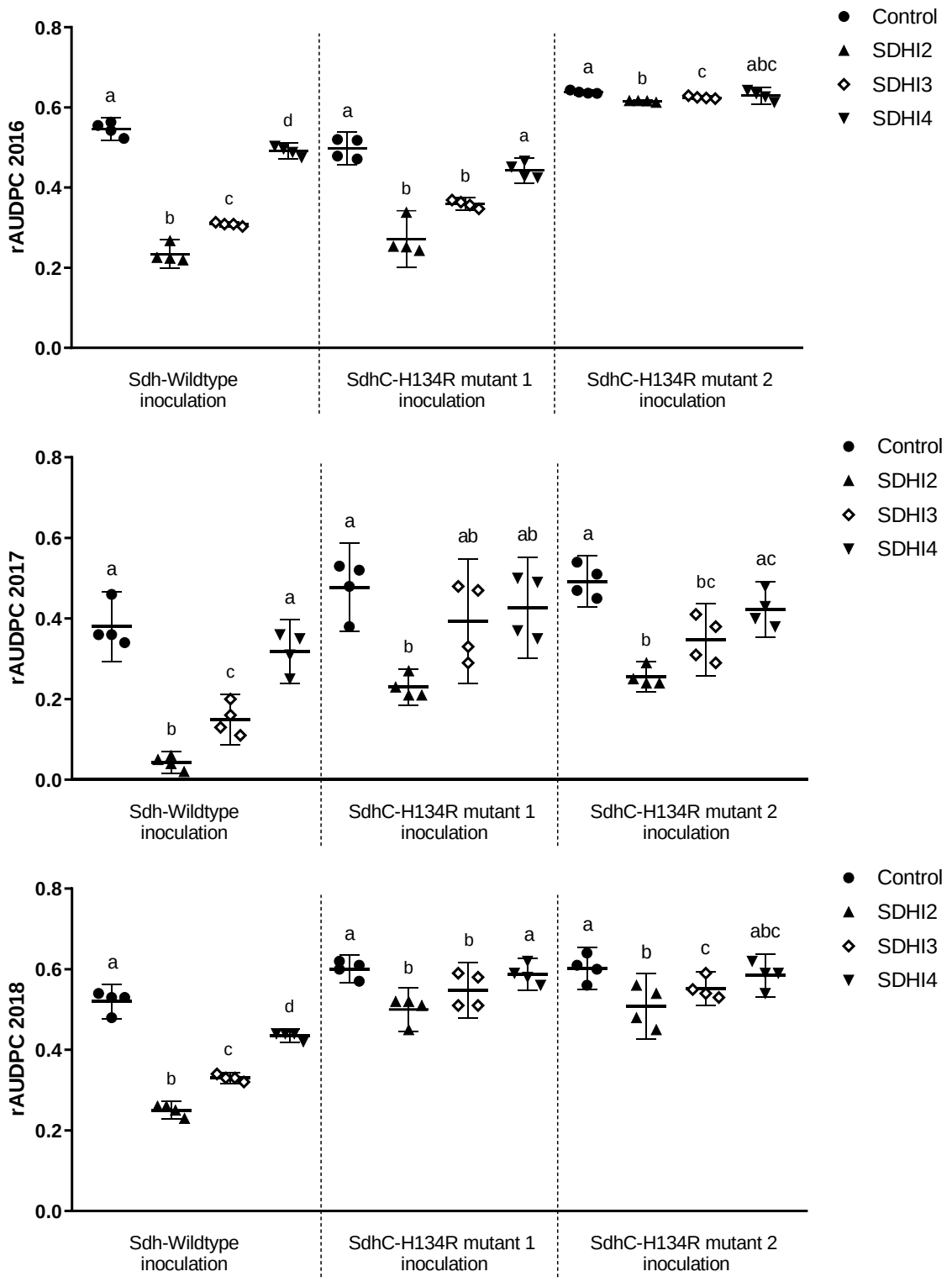

Figure 17 rAUDPC values of different SDHI-fungicide treatments classified according to *Sdh*-wild type, *SdhC*-H134R mutant 1, or *SdhC*-H134R mutant 2 inoculation compared to their untreated controls from field trials 2016 to 2018 with the cultivar Lady Amarilla. Four replicate plots per treatment are shown. SDHI-fungicides with the active ingredients Fluxapyroxad, Fluopyram and Penthiopyrad were tested. The same letters within each inoculation indicate no significant difference according to post hoc Tamhane's T2 test (a=0.05) post hoc test after ANOVA. Error bars indicate 95% CI.

3.3. Impact of Sdh-mutations on fitness

To assess the influence of *Sdh*-mutations on the fitness of *A. solani* isolates, I analyzed six different parameters: spore production capability, spore germination rate, mycelium growth, disease development, and latent period *in vivo* and toxin production.

3.3.1. Spore production capability

To analyze the spore production capability of single *A. solani* isolates, the spore density after two and three weeks of growing on SNA media was determined. In total, 7 (7) *Sdh*-wild type, 7 (5) *SdhB*-H278Y mutant, and 7 (6) *SdhC*-H134R mutant isolates were tested after two (respectively three) weeks of incubation time.

In this experimental setup, no significant difference was detected between the *Sdh*-wild type and *Sdh*-mutated isolates (Fig. 18). A by trend higher spore density of *SdhC*-H134R mutant strains compared to the *Sdh*-wild type and *SdhB*-H278Y isolates was observed after three weeks. A by trend increase of spore density was also found between two and three weeks of incubation time for *Sdh*-wild type (factor 2.08) and *SdhB*-H278Y mutant (factor 1.88) isolates. The *SdhC*-H134R mutant strains showed significantly higher spore densities after three weeks compared to two weeks of growing (factor 2.15). A comparison between spore density of different *Sdh*-regions and incubation times was not performed.

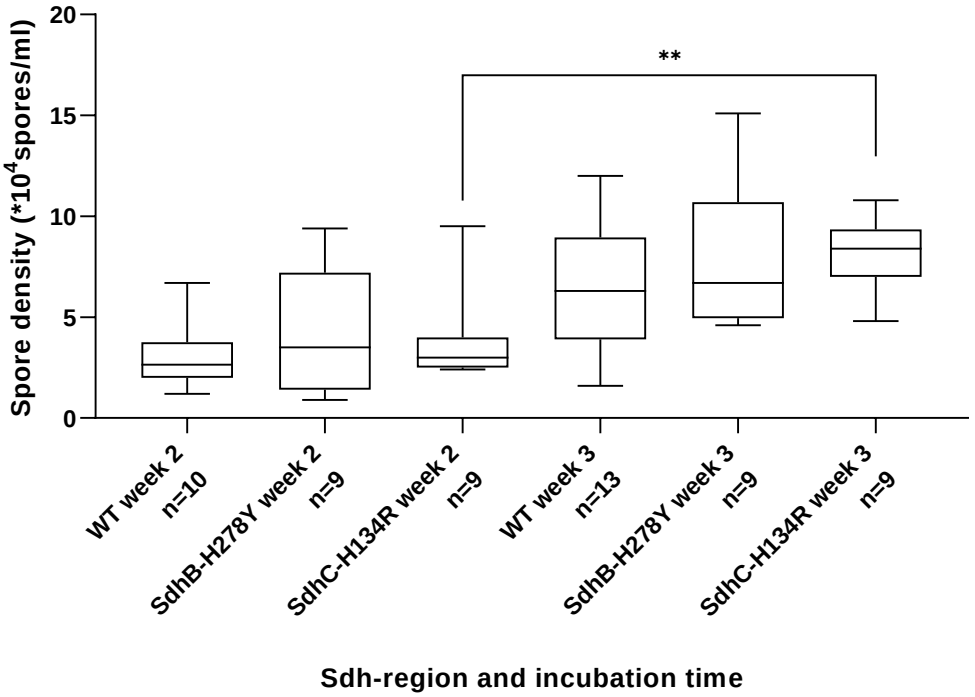

Figure 18 Spore production capability of *A. solani* isolates with different *Sdh*-regions after two and three weeks of incubation on SNA media. In total, 7 (7) *Sdh*-wild type, 7 (5) *SdhB*-H278Y mutant, and 7 (6) *SdhC*-H134R mutant isolates were tested after two (three) weeks. For each isolate and incubation time, one to three plates were analyzed. Significant difference are marked with a star according to Tamhane´s T2 test, post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001*. Whiskers indicate Min to Max. Comparisons between spore density of different *Sdh*-regions and incubation times were not included.

3.3.2. Spore germination

Another fitness parameter is the spore germination rate. In this trial setup, I tested 7 (7) *Sdh*-wild type, 7 (5) *SdhB*-H278Y mutant, and 7 (6) *SdhC*-H134R mutant isolates after two (respectively three) weeks of incubation time.

Overall, no significant difference was detected between the germination rates of the single *Sdh*-genotypes (Fig. 19). A by trend increase of spore germination was observed between two and three weeks of incubation time for *Sdh*-wild type isolates. The *SdhB*-H278Y and *SdhC*-H134R mutant isolates showed significantly higher germination rates after three weeks compared to two weeks of growing. A comparison between the germination rate of one particular *Sdh*-genotype in week two and another *Sdh*-genotype in week three was not performed.

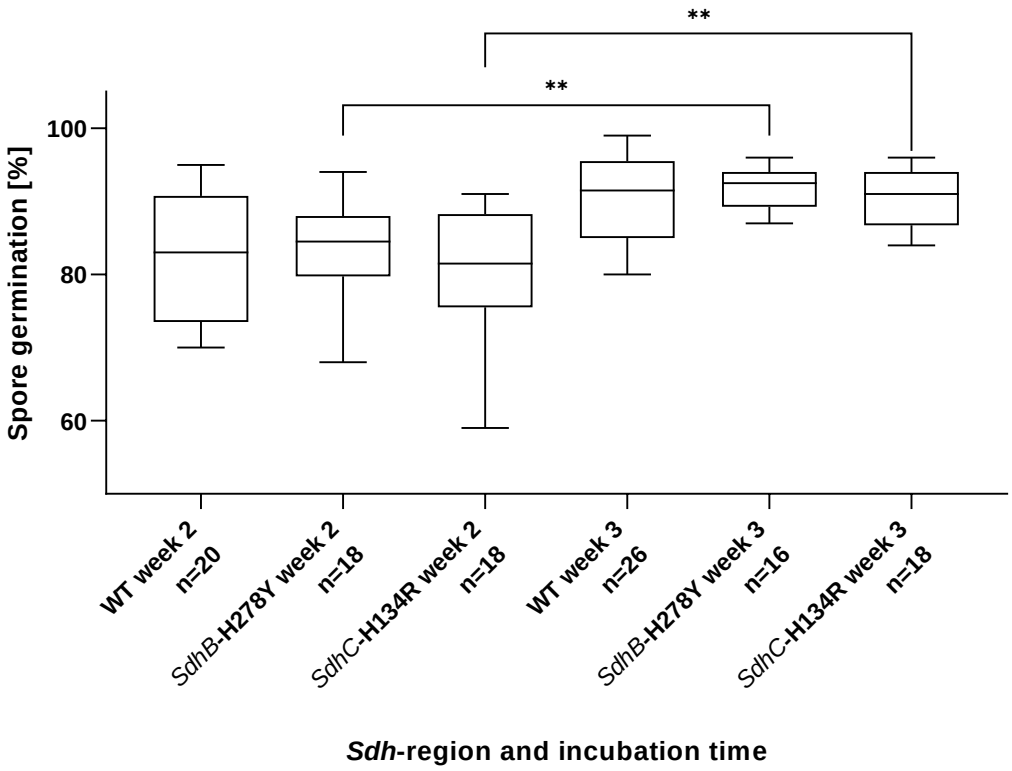

Figure 19 Mean spore germination of *A. solani* isolates with different *Sdh*-regions after two and three weeks of incubation on SNA media. In total, 7 (7) *Sdh*-wild type, 7 (5) *SdhB*-H278Y mutant, and 7 (6) *SdhC*-H134R mutant isolates were tested after two (three) weeks. For each isolate and incubation time, two to four plates were analyzed. Significant difference are marked with a star according to Tamhane´s T2 test post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001*. Whiskers indicate Min to Max. A comparison between the germination rate of one particular *Sdh*-region in week two and another *Sdh*-genotype in week three was not performed.

3.3.3. Mycelium growth

To assess a possible impact of *Sdh*-mutations on the fitness of *A. solani* isolates, the mycelium growth is also one of the parameters we included in our analysis. In total, we measured the radial growth of 8 *Sdh*-wild type, 7 *SdhB*-H278Y mutant, and 6 *SdhC*-H134R mutant isolates after one and two weeks.

After the first week of incubation, a significantly faster growth was measured for *SdhB*-H278Y mutant isolates compared to *SdhC*-H134R mutant strains (Fig. 20). No other differences were visible after one week. This significant difference between the *Sdh*-mutations was still detectable after two

weeks. In addition, the *A. solani* isolates of both *Sdh*-mutations had a significantly faster mycelium growth compared to the *Sdh*-wild type strains. A comparison between the mycelium growth of one particular *Sdh*-region in week two and another *Sdh*-region in week three was not performed.

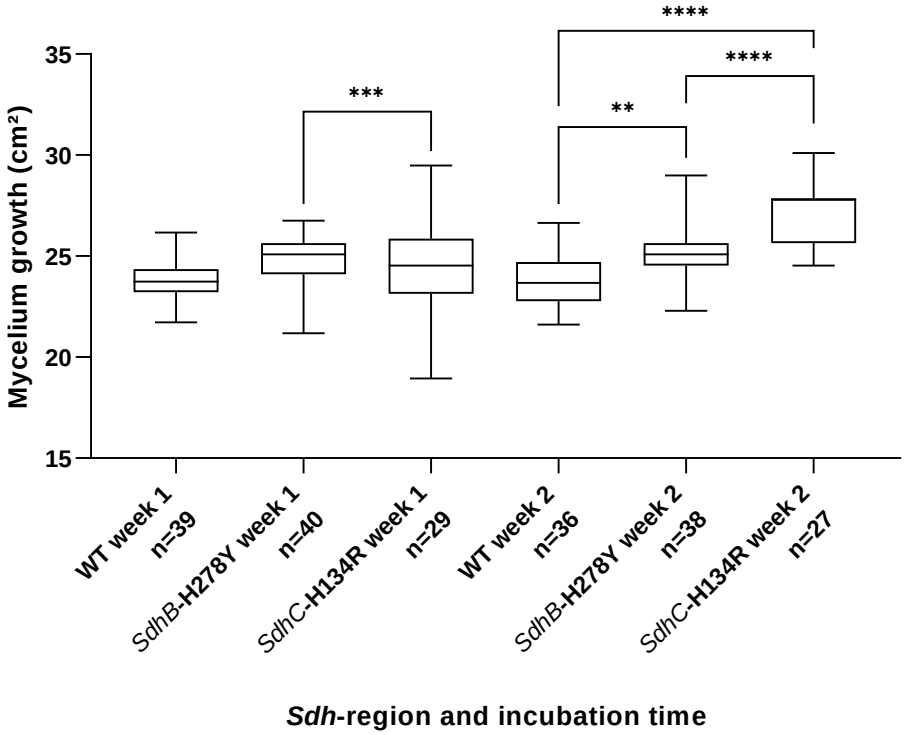

Figure 20 Mycelium growth of *A. solani* isolates with different *Sdh*-regions after one and two weeks of incubation on V8 media. In total, 8 *Sdh*-wild type, 7 *SdhB*-H278Y mutant, and 6 *SdhC*-H134R mutant isolates were measured after one and two weeks. For each isolate, two to eight plates were analyzed. Significant differences are marked with a star according to Tamhane´s T2 test post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001*. Whiskers indicate Min to Max. A comparison between the mycelium growth of one particular *Sdh*-region in week two and another *Sdh*-region in week three was not performed.

3.3.4. Disease development and latent period *in vivo*

We also included the aggressiveness of isolates on plants as a parameter for fitness. Therefore, a greenhouse trial with four *Sdh*-wild type, three *SdhB*-H278Y mutant, and four *SdhC*-H134R mutant strains was performed. For each isolate, three replicate plants were used.

The infected leaf area [%] seven days after inoculation was analyzed, and statistically significant more symptoms were observed for the *Sdh*-wild type isolates compared to both *SdhB*-H278Y and *SdhC*-H134R mutant isolates (Fig. 21). No difference was detectable between the *Sdh*-mutated isolates.

For all tested isolates, first tiny symptoms were visible one day after inoculation. Hence, no influence was observed of *Sdh*-mutations on the latent period.

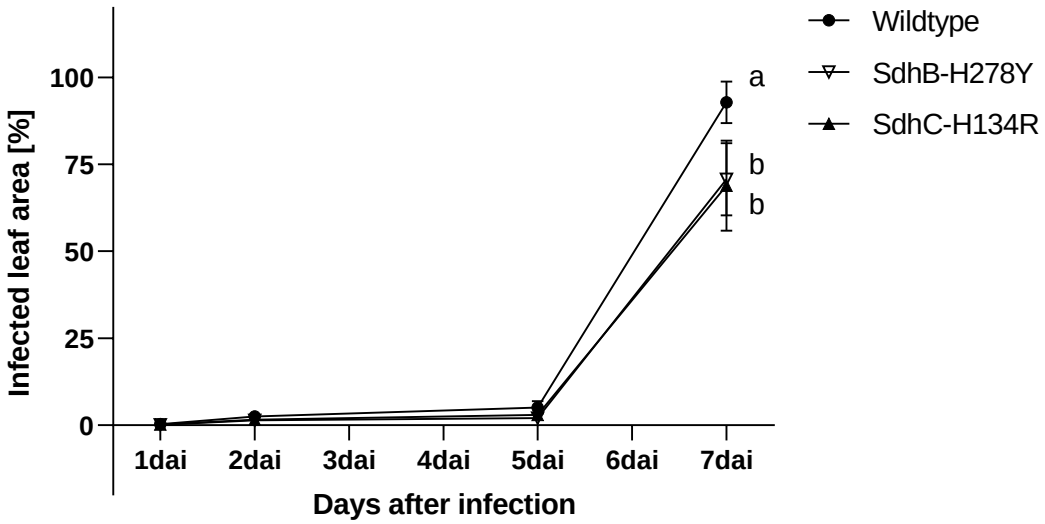

Figure 21 Mean infected leaf area after spray inoculation with either *Sdh*-wild type, *SdhB*-H278Y, or *SdhC*-H134R mutant isolates. Spore density was adjusted to $1x10^4$ spores/ml for all strains. The disease development of four *Sdh*-wild type, three *SdhB*-H278Y, and four *SdhC*-H134R mutant isolates was determined after one, two, five, and seven days. For each strain, three replicate plants were used. This experiment was performed once in greenhouse. No significant differences are marked with the same letter, according to Tamhane´s T2 test post hoc test after ANOVA. P≤0.0061. Error bars indicate 95% CI.

3.3.5. Toxin production

To get a closer look into the toxin production of *Sdh*-wild type and *Sdh*-mutated isolates, we also measured the production of Alternariol (AOH) and Alternariol methyl ether (AME) in liquid culture. In total, 11 *Sdh*-wild type, 8 *SdhB*-H278Y, and 10 *SdhC*-H134R mutant isolates were tested. Each strain was measured three times in collaboration with M. Rychlik from the chair of analytical food chemistry, TUM.

For both AOH and AME, a by trend higher production in the *Sdh*-wild type isolates was visible, but no significant differences were detected (Fig. 22). In general, few strains were observed to produce higher amounts of AOH or AME than others.

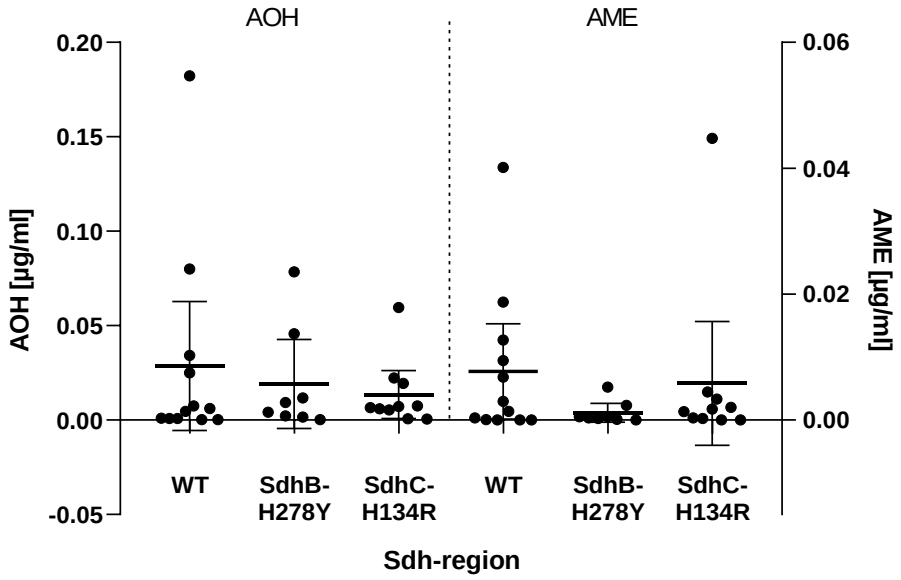

Figure 22 Production of Alternariol (AOH) and Alternariol methyl ether (AME) from different *A. solani* isolates in liquid culture. Each dot represents the average of three measurements of one isolate. In total, 12 *Sdh*-wild type, 8 *SdhB*-H278Y, and 10 *SdhC*-H134R mutant strains were tested. Significant differences are marked with a star, according to Tamhane´s T2 test. Error bars indicate 95% CI. Data provided by M. Gothardt from the chair of analytical food chemistry, TUM.

To answer the question, whether there´s a correlation between AOH and AME production, a Pearson´s r correlation was performed for the isolates within each *Sdh*-group (Fig. 23).

Regarding the *Sdh*-wild type isolates, no correlation between AOH and AME production could be observed (R^2 = 0.01429; P = 0.7114). The isolate, which produced the highest amount of AOH in the *Sdh*-wild type group, was not the same that produced the highest amount of AME. When we compare the production of AOH and AME from the isolates in between the *SdhB*-H278Y mutants, a strong positive correlation was detectable (R^2 = 0.9807; P = <0.0001). The isolate with the highest amount of AOH also produced the most AME. A similar picture is visible for the isolates with the second-highest toxin production. For the isolates with the *SdhC*-H134R mutation, no correlation between the production of both toxins could be observed (R^2 = 0.4132; P = 0.0619). In this group, one isolate produced much higher amounts of both AOH and AME and was therefore excluded from the correlation analysis. Otherwise, the R^2 would be disproportionally strong influenced by one single value.

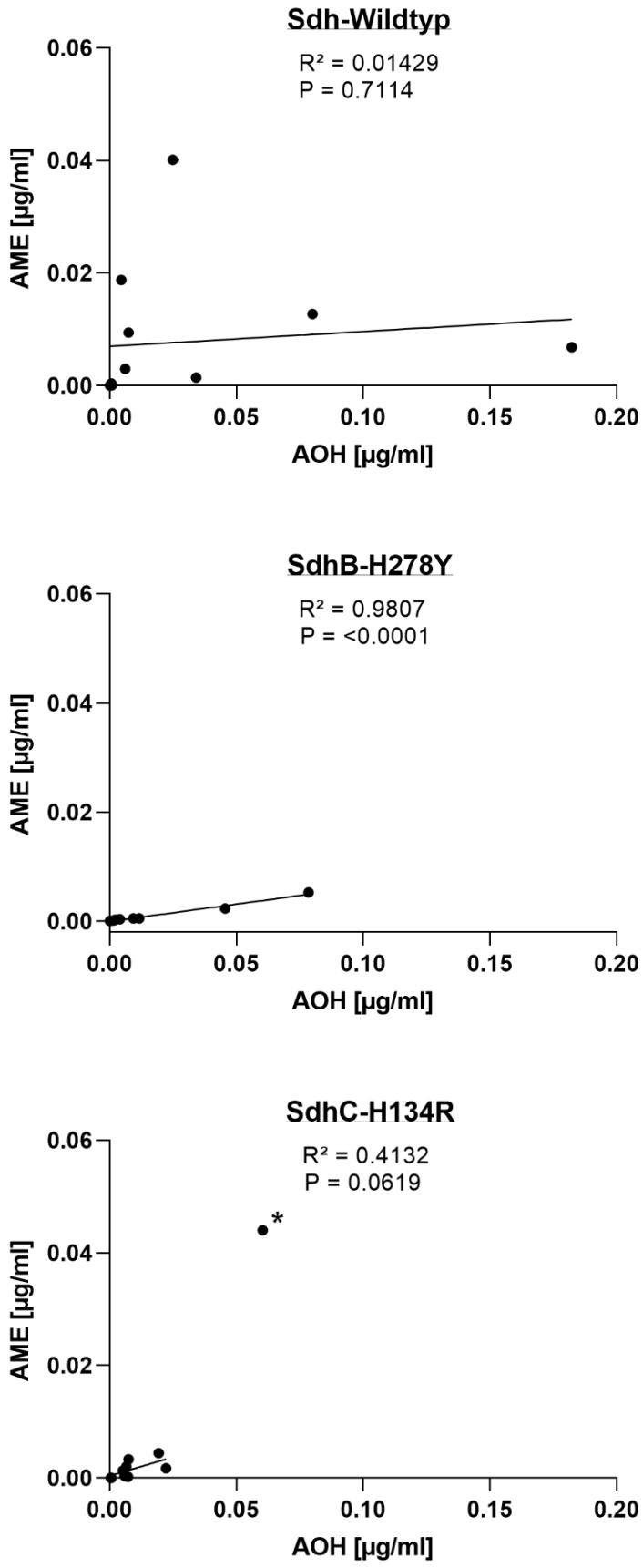

Figure 23 Correlation between AME and AOH levels produced by single isolates in dependency of their *Sdh*-region. In particular, 11 *Sdh*-wild type, 8 *SdhB*-H278Y, and 10 *SdhC*-H134R mutant strains were tested. Outliers are marked with a star and not included in the Pearson´s r correlation analysis.

3.3.6. Overview fitness parameter

As a summary of the results from the fitness tests, we can say that the *Sdh*-mutated isolates showed faster mycelial growth, but were less aggressive in greenhouse trials (Tab. 18). In spore density, spore germination and toxin production, no apparent difference between *Sdh*-wild type and *Sdh*-mutant isolates was observed.

Table 18 Performance of *SdhB*-H278Y and *SdhC*-H134R mutant isolates in comparison to the *Sdh*-wild type isolates. Fitness parameters like mycelia growth, spore density, spore germination, production of AOH/AME, and disease progression were included. The arrows indicate higher (↑), similar (→), or lower (↓) performance compared to the *Sdh*-wild type isolates.

Fitness parameter	***SdhB*-H278Y**	***SdhC*-H134R**
Mycelia growth	↑	↑
Spore density	→	→
Spore germination	→	→
Production of AOH/AME (liquid culture)	→	→
Disease progression (greenhouse)	↓	↓

3.4. Trichoderma spp. as Biological Control Agents

To evaluate the potential of *Trichoderma* as a BCA against *A. solani* on potato, I used three different testing systems: *in vitro* dual culture tests, greenhouse trials, and field trials. Six *Trichoderma* strains were provided by Laszlo Kredics from the Szeged University in Hungary.

3.4.1. Efficiency of *Trichoderma* spp. against *A. solani in vitro*

In the first step, the capability of *Trichoderma* to inhibit *Alternaria* growth on plates was studied *in vitro*. Therefor a dual-culture test was performed with three *A. solani* isolates and six *Trichoderma* spp. isolates. In detail, two *T. harzianum*, two *T. atroviride*, one *T. hamatum,* and one *T. asperellum* isolates were used. The growth inhibition was calculated after five days of incubation time.

Overall, each *Trichoderma* isolate was able to reduce *A. solani* growth on plates (Fig. 24). The inhibition rate varied between 35 and 85%. On average, the two *T. atroviride* isolates (66 and 65%) showed by trend stronger growth inhibition than *T. hamatum* (54%) and *T. asperellum* (55%). A significantly higher reduction in growth was observed for both *T. atroviride* compared to both *T. harzianum* isolates (50 and 35%). *T. harzianum* 20770 indicated the weakest growth inhibition (35%) and showed significant differences compared to all other tested strains. Overall, there were only minor differences between the inhibitions of the tested *A. solani* isolates, so they were pooled together for the analysis.

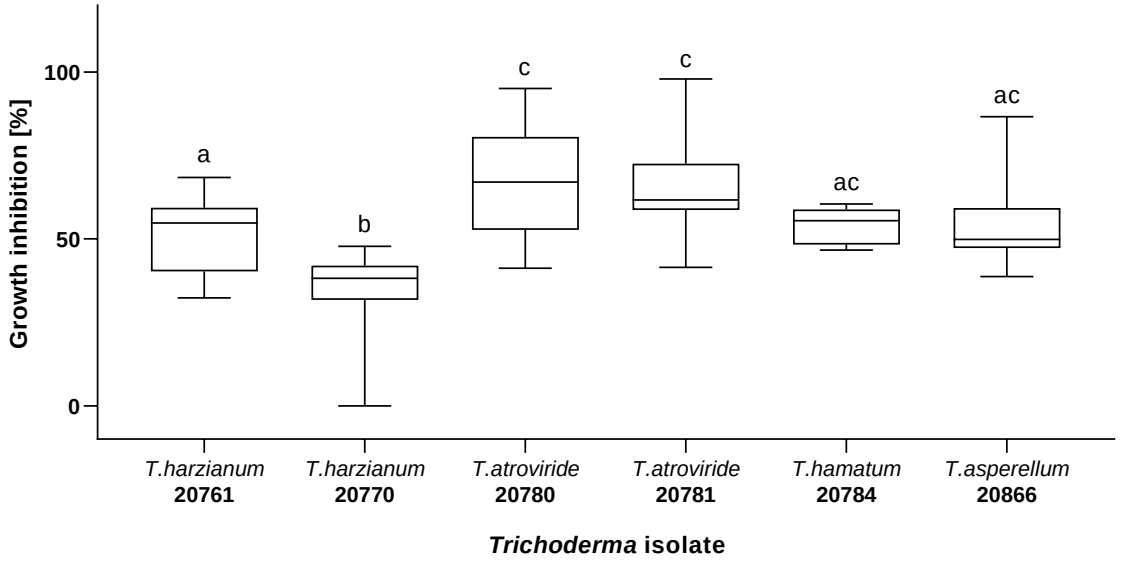

Figure 24 Growth inhibition potential of *Trichoderma* spp. *in vitro*. In total, a dual culture test was performed with three *A. solani* and six *Trichoderma* isolates. Each boxplot includes the measurements of all three tested *A. solani* isolates against the particular *Trichoderma* strain (n=15). Growth inhibition [%] was calculated after five days of incubation time. For each *A. solani* isolate, five replicate plates per *Trichoderma* isolate were tested. Boxes marked with the same letter are not significantly different according to Post hoc Tukey test (α=0.05). Whiskers indicate Min to Max.

3.4.2. Efficiency of *Trichoderma* spp. against *A. solani in vivo*

In the next step, the efficiency of spore solutions from the different *Trichoderma* isolates as BCAs against early blight infection was tested in greenhouse. In detail, two *T. harzianum*, two *T. atroviride*, one *T. hamatum,* and one *T. asperellum* isolates were used for treatments one day before *A. solani* inoculation. For each treatment, three replicate plants were included and the whole setup was repeated twice. The multisite fungicide Chlorothalonil served as a positive control.

Overall, the efficiency varied between 15 and 57% (Fig. 25). The *T. harzianum* 20761 isolate showed the highest efficiency with 57% and performed significantly better than the *T. harzianum* 20770 isolate with 15%. A significant difference was also observed between the *T. harzianum* 20770 and *T. asperellum* 20866 strain (about 47%). A by trend higher efficiency of *T. asperellum* was visible compared to the other tested isolates, except *T. harzianum* 20761. *A. solani* infection was inhibited by 99% after treatment with Chlorothalonil.

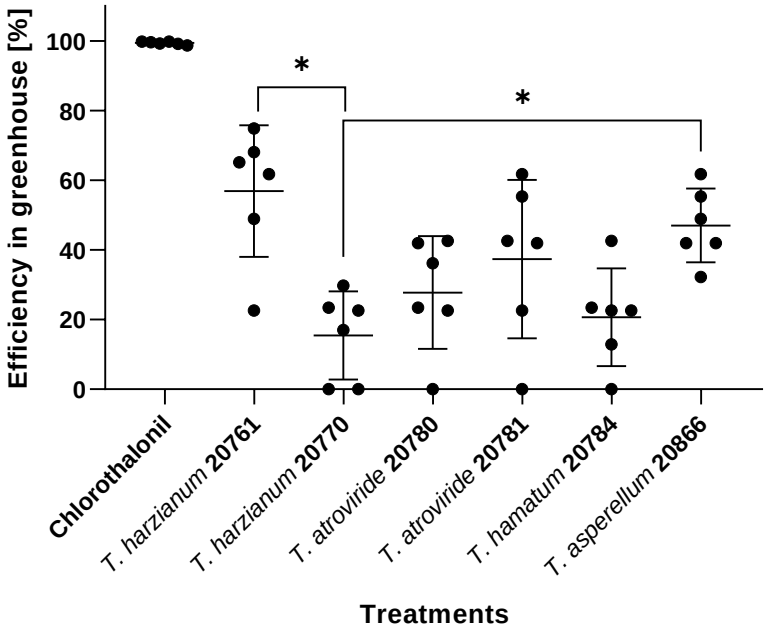

Figure 25 Efficacy of spore solutions from different *Trichoderma* isolates in greenhouse trials compared to an untreated control. Spore density of *A. solani*: $1x10^5$ spores/ml and *Trichoderma* spp.: $5x10^7$ spores/ml. For each treatment, three replicate plants were used. The whole setup was repeated twice. Significant differences are marked with a star according to Tamhane´s T2 test post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001*. Error bars indicate 95% CI.

3.4.3. Efficiency of *Trichoderma* spp. against *A. solani* in the field

In the final step, the efficiency of *Trichoderma* spore solutions, as a BCA against early blight infection, was tested under field conditions between 2016 and 2019. The weather conditions for these four years are described in chapter 3.2.4.

The disease development varied in each year (Fig.26). In 2016 and 2017, the first symptoms appeared end of July/beginning of August. In contrast, in the following two years, the first symptoms were observed end of June/beginning of July. In 2016, 2018, and 2019, around 100% infected leaf area was reached in the untreated controls. In 2017, a disease progression of about 25% was measured at the end of the season. The 100% infection level was reached end of August for the years 2016 and 2019 and end of June in 2018.

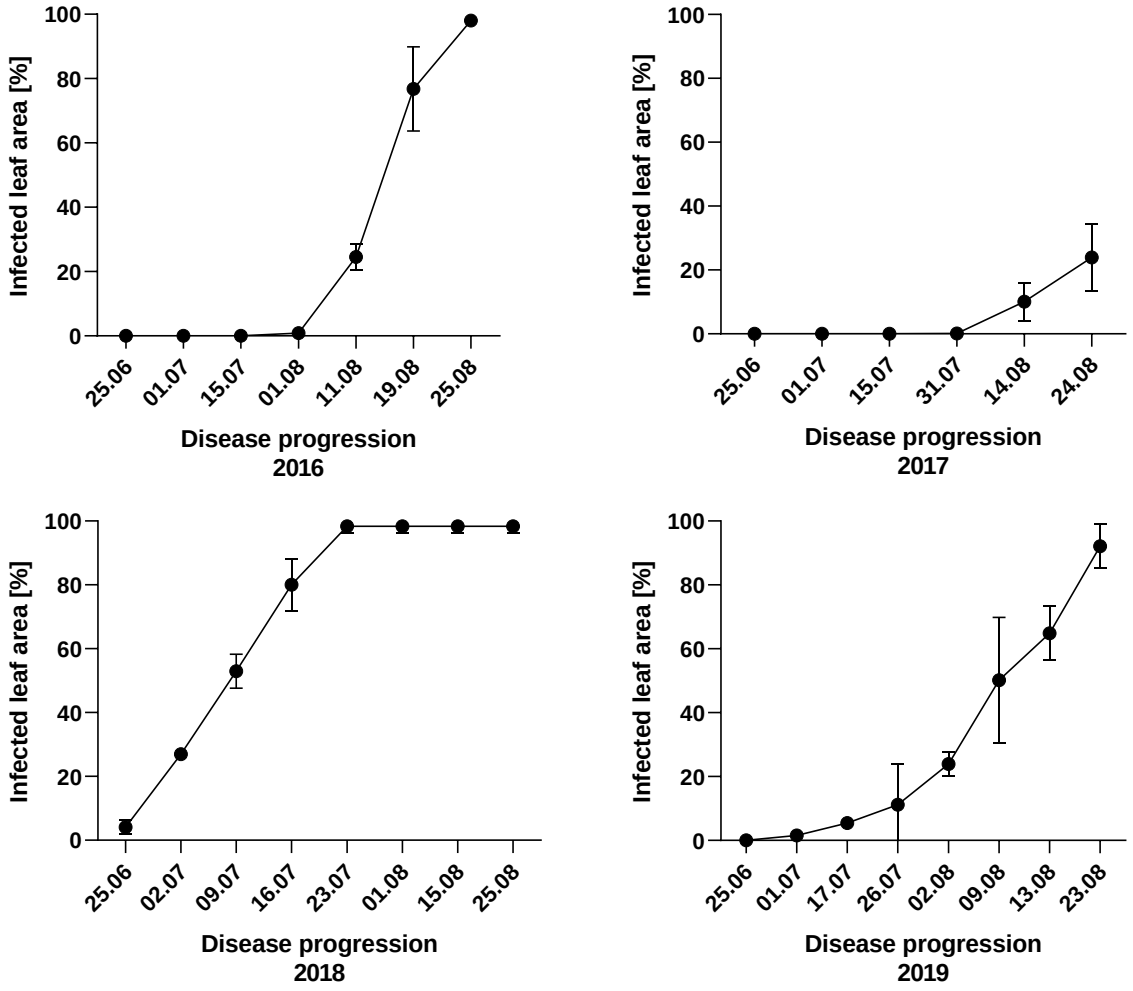

Figure 26 Disease development of the untreated controls between 2016 and 2019. Mean out of four replicate plots is displayed. Error bars indicate 95% CI.

This high variation of the disease development leads to the decision to analyze each year separately in the following paragraphs.

For the analysis of the *Trichoderma* field trials I choose two different approaches. In a first step, I compared rAUDPC values of each treatment with the rAUDPC value of the untreated control (Fig. 27). In a second step, I decided to display the efficacy of the treatments at one specific time point. This time point was chosen individually for each year, according to the start of the progression-phase in the untreated control (at least 20% disease severity) (Fig. 28).

In 2016, the treatments with *T. harzianum* 20761, *T. atroviride* 20780, *T. atroviride* 20781 and the fungicide with its active ingredient Chlorothalonil significantly decreased the rAUDPC values compared to the untreated control. Regarding the rAUDPC values in 2017 and 2019, I couldn´t detect any significant differences between the treatments (including the fungicide) and the untreated control. In 2018, only the Chlorothalonil treatment led to a significant reduction of the rAUDPC in comparison to the untreated control.

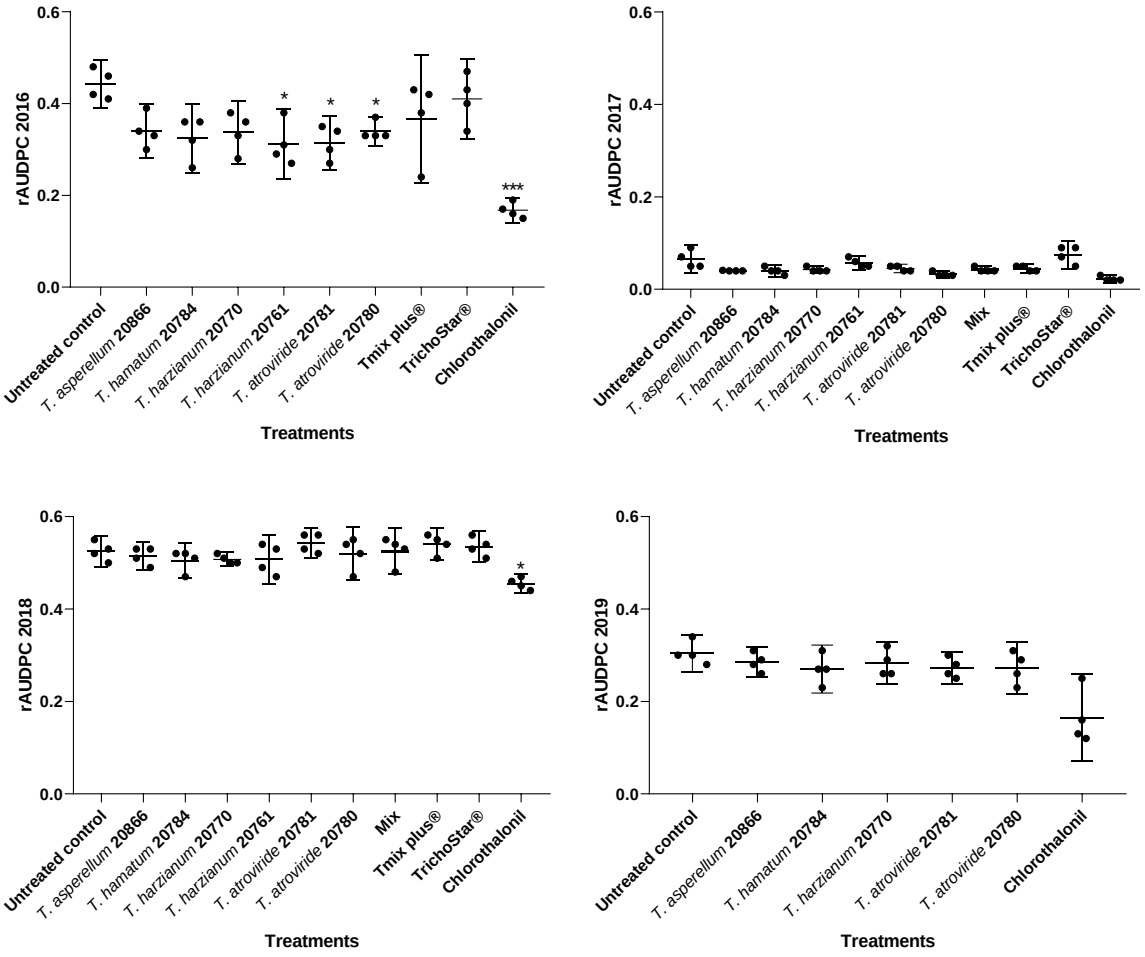

Figure 27 rAUDPC values of the *Trichoderma* treatments with spore solutions and commercial products conducted in the years 2016 to 2019. Four replicate plots per treatment are shown. Treatment "Mix" implements a mixture of *T. harzianum* 20761, *T. atroviride* 20781 and *T. hamatum* 20784. Chlorothalonil serves as a fungicide control. Not every treatment was included in each year. Significant difference between a treatment and the untreated control is marked with a star according to Tamhane´s T2 test, post hoc test after ANOVA. **=P≤0.05; **=P≤0.01; ***=P≤0.001; ****=P≤0.0001*. Error bars indicate 95% CI. Comparisons between each of the treatments were not included.

To get a better picture of the efficacies of the *Trichoderma* treatments, I wanted to analyze the impact of the treatments in an earlier stage with moderate disease pressure. Therefore, I chose the time point with around 20% disease severity in the untreated controls for each year (Fig. 28).

In 2016, the displayed time point is 11.08.16. Most of the treatments (except TrichoStar®) showed an efficiency of about 50%. The effectiveness of the fungicide treatment was around 60%.

In 2017, the efficacy on the 24.08.17 is shown. For *T. harzianum* 20770, *T. atroviride* 20780, *T. hamatum* 20784, and our own *Trichoderma* mixture, around 20% efficiency was observed. The treatments with *T. atroviride* 20781, Tmix plus®, and TrichoStar® lead to a disease reduction of under 10%. *T. asperellum* 20866 treatment showed an efficacy of about 25%. The efficiency of the fungicide treatment was around 50%.

For the year 2018, the time point 02.07.18 is shown. Regarding the *Trichoderma* treatments, *T. asperellum* 20866 and *T. harzianum* 20770 lead to a reduction of early blight infection of about 5%. All other treatments, including the fungicide, showed no reduction.

In 2019, the displayed time point is 02.08.19. The treatments with *T. harzianum* 20770, *T. asperellum* 20866, *T. hamatum* 20784 and *T. atroviride* 20781, lead to a decrease in early blight infection of about 10%. *T. atroviride* 20780 showed a nearly 20% reduction compared to the untreated control. The fungicide treatment showed about 40% efficiency.

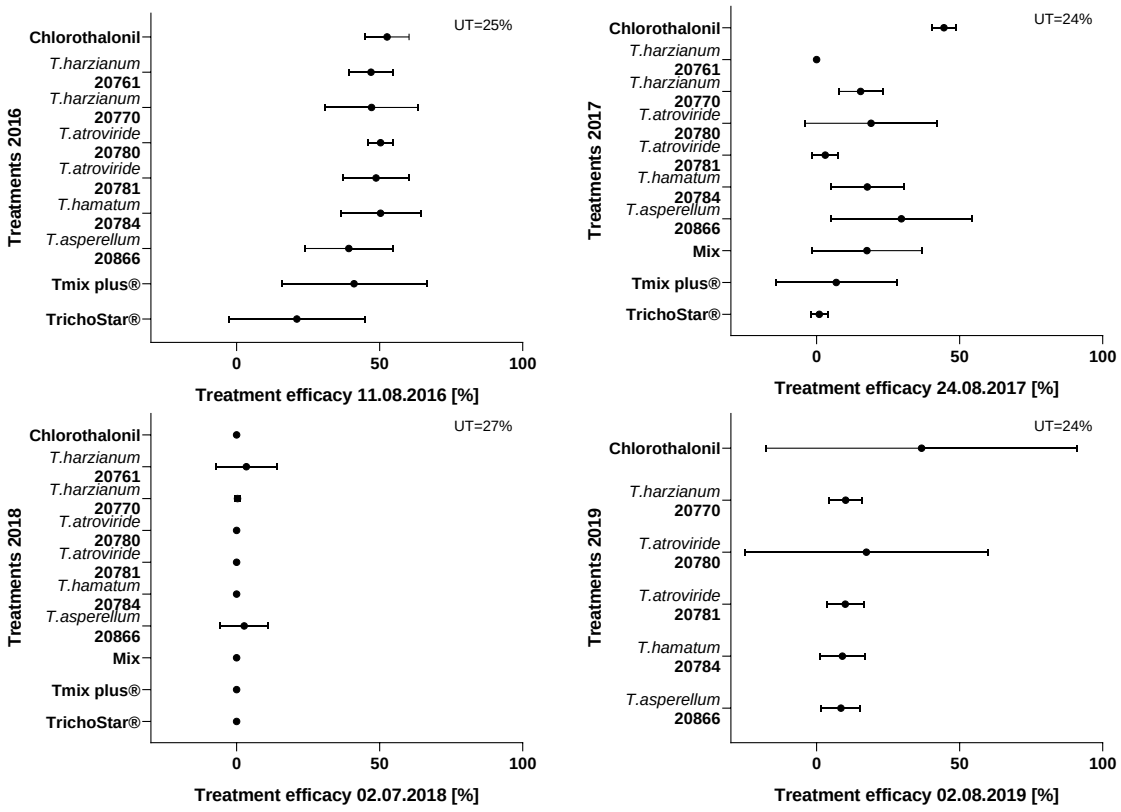

Figure 28 Efficiency of different treatments for one specific time point of the years 2016 to 2019. Displayed time points were chosen individually for each year according to the disease level in the untreated control (at least 20%). Treatment "Mix" implements a mixture of *T. harzianum* 20761, *T. atroviride* 20781 and *T. hamatum* 20784. Chlorothalonil serves as a fungicide control. Not every treatment was included in each year. Efficiency was calculated for each replicate plot compared to the mean of the untreated control plots. For each treatment four replicate plots were included. UT=disease severity in untreated control. Error bars indicate 95% CI.

4. Discussion

Early blight is one of the main diseases of potato worldwide. Yield losses of 1 to 60% are reported from several different countries, e.g., Germany, the United States, South Africa, or Australia (Harrison and Venette 1970; van der Waals et al. 2003; Horsfield et al. 2010; Leiminger and Hausladen 2014). As for most pathogens, the weather conditions profoundly affect the disease severity, which then results in varying yield losses. Delgado-Baquerizo et al. (2020) recently predicted that the proportion of soil-borne pathogens like *Alternaria* spp. will clearly increase with future global warming. This together with the rapid development of fungicide resistance by *A. solani* motivated further in depth

studies for better understanding of how the disease develops and how chemical and complementary biological plant protection measures might work in future.

To get more in-depth knowledge about the epidemiology and the behavior of *A. solani* under changing environmental conditions, I analyzed several parameters during this study: pathogen isolates from alternative hosts, temperature, latent period, and leaf moisture. In addition to these epidemiological studies, the sustainable and sufficient control of early blight was also one main topic of this research. As resistance development is already reported against QoI fungicides for a relatively long time (Pasche and Gudmestad 2007; Leiminger et al. 2014), I focused on SDHIs. To get more profound knowledge about the presence of *Sdh*-mutated *A. solani* strains in Germany, samples from different locations in Germany were gained and sequenced from 2013 to 2016. For assessing the possible consequences of rising numbers of mutations, I evaluated the sensitivity of *A. solani* isolates in three different testing systems and a survey of *Sdh*-mutated strains for potential disadvantages in fitness was included. In addition to chemical disease control, I also studied the potential of *Trichoderma* as a possible BCA. Therefor I tested the ability of *Trichoderma* strains to control *A. solani in vitro*, *in vivo,* and in the field. In the following, the parts of this study mentioned above will be discussed separately in detail.

4.1. Epidemiology of early blight of potato

According to Olesen et al. (2011), extreme weather events will increase with climate change (e.g., heavy rainfall, heat periods), and Delgado-Baquerizo et al. (2020) clearly stated that the proportion of soil-borne pathogens like *Alternaria* spp. will increase with global warming in future. In this context, I wanted to know more about the behavior of *A. solani* under changing environmental conditions. In detail, I wanted to gain more profound knowledge about the infection conditions for *A. solani* on potato. Therefore, several greenhouse trials were conducted to answer two main questions: How do different temperature conditions influence the disease development on leaves, and how long does the period of leaf wetness have to be for a successful infection?

In our experiments, three different temperatures were included: 18, 22, and 26°C. The infection conditions directly after inoculation were the same for all plants to ensure comparable infection rates at the beginning. For inoculation two different *A. solani* strains were used separately. In general, a much more substantial increase in disease severity was observed on the plants at 26°C, compared to 18 and 22°C. This picture was similar for both tested *A. solani* isolates. In the second setup, one strain showed hardly any differentiation between the individual temperatures. Because the disease level was lower for all treatments with this isolate compared to the first repetition, this indicates that there was a problem with the proper infection itself. One possible explanation might be less aggressive spores compared to the first repetition, or the conditions during the incubation phase in the tent were not ideal for infection. However, it seems like rising temperatures increase the severity of early blight disease progression. One reason for the high severity of the disease could be an increasing toxin production of the pathogen under higher temperature conditions. Indeed, Pose et al. (2010) showed a rising mycotoxin production (AOH/AME and TA) for *Alternaria alternata* on tomato pulp agar when cultivated at 21°C compared to 6 or 15°C. In contrast to that, Özcelik and Özcelik (1990) analyzed the ability of produced metabolites by *A. solani* in liquid culture at 6, 15, and

25°C to inhibit bacterial growth on plates. In this study, a growth inhibition was only visible for the liquid media produced by *A. solani* at 15°C. Even though Özcelik and Özcelik (1990) worked with the same species as I did, they only used one isolate for their comparison and according to my toxin production studies, the variability between strains from the same species can be quite high. Therefore, further research is needed to analyze the toxin production of *A. solani* under different temperature conditions in more detail – especially in direct contact with the host plant. Besides secondary metabolites, the production of biomass can also give some hinds on the effect of different temperature conditions on *A. solani*. Özcelik and Özcelik (1990) also showed a clear trend for higher biomass production in liquid media for *A. solani* with rising temperatures (6, 15, 25°C). In addition, Somappa et al. (2013) observed an increasing mycelial growth and sporulation of *A. solani* on PDA medium with rising incubation temperatures up to 25°C. These findings also support the assumption that higher temperatures promote infection with *A. solani*. Another possible explanation would be that the plants were more stressed at 26°C compared to 18 and 22°C and this lead to an increase of susceptibility to early blight. It has been shown that potato plants not only develop differently under heat stress (Ben K. M. and Ewing 1985), but also the expression of single host genes and fungal toxins is influenced (Heat shock proteins, pathogenesis-related proteins, AOH and AME) (Pose et al. 2010; Makarova et al. 2018). However, a direct effect hasn´t been shown so far of heat stress on the susceptibility against early blight on potato, and it remains also unclear if 26°C is indeed stressful for potato plants. Nevertheless, this is a severe problem for agriculture, because a clear trend of increasing numbers of summer days (with at least 25°C) has been reported during the last years e.g. for Bavaria, Europe or China (Wei and Chen 2011; Bayerisches Staatsministerium für Umwelt und Verbraucherschutz 2015; Dong et al. 2017). That means, more research is needed – both on the pathogen and on the plant side – to answer the question why we observe higher infection rates with rising temperatures.

Not only temperature but also the availability of free water on the leaves is crucial for a successful infection of potato by *A. solani*. The climatic changes may provoke the hypothesis that there´ll be less leaf moisture, because of the rising temperatures and decreasing amount of rainfalls. However, only a part of leaf wetness can be explained with precipitation according to the correlation analysis for the years 2016 to 2019 (R^2s below 0.1). On the other hand, I could find a medium correlation between leaf wetness and temperature in those years (R^2s between 0.18 and 0.3). These medium R^2 values might be explainable by the day/night rhythm, because during the night we have lower temperatures and in many cases dew formation. However, I could observe a relatively high and significant correlation between leaf moisture and for all years (R^2s of 0.41 to 0.63). Because of this relatively strong correlation between RH and free water on the leaves, I also analyzed the influence of precipitation on RH. For the years 2016 to 2019, R^2s under 0.04 were calculated. This indicates that we cannot assume future reduced leaf moisture due to rising temperatures or decreasing precipitation. Overall, leaf wetness is complex, and many parameters interfere with each other and influence leaf moisture to a certain degree. However, these findings lead to the next question addressing the minimum period of leaf wetness for a successful infection of *A. solani* on potato. During my greenhouse trials, the appearance of the first symptoms started one day after infection. These observations confirm the notes from Vloutoglou and Kalogerakis (2000), who recorded the

first symptoms of *A. solani* on tomato 24h post-inoculation. These findings were the basis of the setup for the leaf moisture duration experiment. Periods of 6, 8, 10, 12, 16, 18, 20, 22, and 24h were tested. The key message from this study is that *A. solani* can infect potato plants from the cultivar Kuras after a minimum leaf wetness period of 8 hours. In 2000, Vloutoglou and Kalogerakis studied the effects of leaf wetness on *A. solani* infection on tomato and observed a cultivar dependent pattern. For one cultivar, 4 hours of leaf wetness were sufficient for successful infection, and for another cultivar, 6 hours were needed. In my trial with the cultivar Kuras, 6 hours of leaf wetness were not enough for successful infection. Comparable results were shown from Bashi and Rotem (1974) with *A. porri f. sp. solani neerg* on potato. Successful infection was detectable with 8 hours of leaf moisture (split into two periods of four hours and a twelve-hour break in between) at a minimum temperature of 15°C. Taking into account our results and the notes from literature, we can conclude that 8h of free water seems to be sufficient for the fungi to infect its host. Now the question arises whether conditions can be found in the field that are comparable to those in my experimental setup. To answer this question, I took a closer look into the periods of leaf moisture, which occur mainly during the night, even if there´s no rainfall. First, I had to find a suitable indicator for leaf wetness. Sentelhas et al. (2008) compared three different methods for estimation of leaf wetness. They concluded that the model based on relative humidity (≥90%) performed best, but still had some issues with underestimating leaf wetness. Kruit et al. (2008) also showed good results with RH (≥87%) as an estimator for free water on leaves. To minimize overestimation, I decided to use two scenarios for RH: ≥90 and ≥95%. Besides the availability of free water, temperature also plays an important role for successful infection of *A. solani*. Therefore, I wanted to include a minimum temperature as an additional parameter in my analysis. It was shown that *A. porri f. sp. solani neerg* was able to infect potato plants at 15°C (Bashi and Rotem 1974). In studies with *A. solani* on potato, *A. linicola* on linseed, *A. brassicae* on oilseed rapes and *A. cirsinoxia* on Canada thistle, leaves got infected at 10°C (Waggoner and Horsfall 1969; Hong et al. 1996; Vloutoglou et al. 1996; Green and Bailey 2000). Based on literature, I hence decided to use again two scenarios – one with ≥10°C and the other with ≥15°C. With these specifications for RH and temperature, we screened for periods of at least 8h with ≥90 or ≥95% RH and simultaneously ≥10 or ≥15°C between May and August for the years 2016 to 2019. This analysis clearly shows that even if there was not much rain anymore, the conditions were still sufficient for an infection with *A. solani* during the potato growing season and that we have several possible infection periods distributed over the potato season. If we compare the occurrence of the favorable conditions - regardless of the individual climatic scenarios - with the observation of the first symptoms, it is noticeable that the first symptoms do not appear on the plants immediately after the first favorable day. This observation holds true for all four tested climatic scenarios. This could be an indication that *A. solani* needs several such periods to infect finally the plant successfully, but further research is needed to confirm this. Besides the naturally occurring leaf moisture formation, we also have to keep in mind that potato fields are often irrigated by farmers. This also generates longer periods of leaf wetness. Together, this knowledge needs to be taken into account when we think about controlling the pathogen and guarding the yield.

Another point that needs to be considered, when we talk about managing early blight, is the possible function of diverse alternative host plants as an additional inoculum source of *A. solani*. It is not only

necessary to know which plants can be infected – but it´s also crucial to understand if isolates from those hosts can infect potato plants and cause early blight disease. I therefore studied *A. solani* strains from several different host tissues and plants: potato leaf, potato tuber, tomato (*Solanum lycopersicum*), wild tomato, black nightshade (*Solanum nigrum*) and thistle (*Cirsium arvense*). This demonstrated that all tested *A. solani* strains were able to infect potato plants under greenhouse conditions. The latent period was one day for all isolates, and no significant differences were observed between the disease severities caused by those strains.

Potato tuber infection is not very common, but isolates gained from tubers can infect potato plants again. This is not only a problem regarding the inoculum source on the field, but also for seed potatoes. Most farmers buy their seed potatoes from specific companies, and on that route, *A. solani* isolates could be easily distributed. Especially the spread of mutated fungicide-resistant genotypes is an issue here. Hence, the production of healthy planting material is a key factor for less infection on each field and for slowing down the spread of mutated isolates. Tomato and wild tomato belong to the family of *Solanacea* together with potato and black nightshade. This makes it not very surprising that the strains isolated from these hosts are able to infect potato plants. Zahur (1985) also showed the ability of *A. solani* to infect several different plant species, which all belong to the family of *Solanacea*. It´s essential to keep that in mind to minimize all sources of inoculum in and around potato fields. Besides black nightshade, it was shown in this study that *A. solani* isolated from thistles can also infect potato plants and thus serve as additional inoculum source. Akhtar et al. (2011) observed the ability of *A. solani* (from tomato) to infect field winch (*Convolvulus arvensis*). The function of weeds as alternative hosts for *A. solani* is especially important in the context of field sanitary and weed control. So even if there´s no potato on the field in one year, weeds can support the reproduction of inoculum as well.

In the frame of the analysis of strains from diverse hosts, I also assessed their ability to produce spores and their germination rate to find differences between isolates. No correlation could be detected between spore density or spore germination and the origin from a specific host. However, a strong correlation was observed between the number of produced spores and the germination rate – independent from the original host. It seems like a high number of spores is associated with a lower germination rate and *vice versa*. That could imply that *A. solani* isolates may have two different strategies to reproduce, which could be mutually exclusive. Either they produce a lot of spores, which are not as vivid as others, or they provide a smaller amount of spores, which are highly vivid. These interesting data need to be confirmed for other isolates. So far, it´s only shown in literature that a higher spore concentration of the same strain leads to self-inhibitory effects on the germination rate (Louis et al. 1988; Caipo et al. 2002), but I adjusted spore solutions to the same spore concentration, so that this can´t be the reason for varying rates of spore germination.

4.2 SDHI resistance in A. solani

Fungicide resistance is one of the major problems in chemical plant protection worldwide and represents a high risk for global food security (Fisher et al. 2018; Fones et al. 2020).

Beside QoIs and DMIs, the SDHIs are one of the most frequently used fungicide groups, which are specific for early blight in potato. Hence, the selection for mutated isolates is often the consequence of the extended use. In many cases, the occurrence of mutated genotypes is associated with a reduced QoI- or SDHI-fungicide sensitivity tested *in vitro* and in greenhouse trials (Leiminger et al. 2014; Landschoot et al. 2017a; Rehfus et al. 2018). However, direct evidence is lacking that *A. solani* field isolates with *Sdh*-gene mutations interfere with the efficacy of SDHI fungicides in the field. To get a general overview of the occurrence and distribution of *Sdh*-mutated *A. solani* isolates in Germany, a monitoring was conducted between 2013 and 2016. Therefore, many isolates were collected and sequenced for their *Sdh*-genes and indeed, several different mutations have been found: *SdhB*-H278R, *SdhB*-H278Y, *SdhC*-H134R, and *SdhD*-D123E. These mutations were all previously described by (Mallik et al. 2014). In addition to the known mutations, a new genotype has been detected: *SdhC*-H134Q (Metz et al. 2019). The *SdhC*-H134Q mutation was also found in *Didymella tanaceti* by Pearce et al. in 2019. Similar to the study from Pearce et al. (2019) with Tasmanian *D. tanaceti* isolates, the frequency of this new mutation was quite low in German *A. solani* isolates, and it was only found in 2016 and 2017. This lower frequency may be explained by the fact that *SdhC*-H134Q mutant isolates didn´t show a strong increase in SDHI resistance – hence these mutants might have been outcompeted by less SDHI-sensitive strains, e.g., *SdhB*-H278Y and *SdhC*-H134R mutants. Pearce et al. (2019) also showed a higher sensitivity of the *SdhC*-H134Q mutant strain towards boscalid compared to other – more frequently appearing – mutant strains, like SdhB-H277Y, *SdhB*-H277R and *SdhC*-H134R. In general, the amount of *Sdh*-mutations continuously increased in Germany over time. Bauske et al. (2018a) found the *SdhB*-H278Y mutation to be predominant in 2011 and 2012, whereas the *SdhC*-H134R mutation was most frequent in 2015 in the United States. Unlike to the US, in Germany, the *SdhC*-H134R mutation was the most frequent mutation in all analyzed years.

To get a clearer picture about the impact of the individual *Sdh*-mutations on the disease control, the fungicide efficacy of one of the most frequently used fungicides to control early blight – Cantus® and its active ingredient boscalid – was tested in different systems (Metz et al. 2019). To broaden the understanding, additional SDHI-fungicides were included in the *in vitro* and field experiments. In all tested systems, the presence of frequent *Sdh*-mutations led to decreased fungicide efficacy, but differences were observed between active ingredients of the SDHI-group. For most of the tested isolates with an *SdhB*-H278Y or *SdhC*-H134R mutation, a highly reduced sensitivity against boscalid was determined *in vitro* (EC_{50} >20µg/ml). Only few mutated strains were categorized as moderately resistant (EC_{50} 5-20µg/ml), and only one *SdhC*-H134R mutant isolate showed a sensitive response to boscalid. These findings are comparable with those of Gudmestad et al. (2013) and Landschoot et al. (2017a). They also found more highly resistant isolates compared to moderately resistant strains in some parts of the United States and Belgium, respectively.

In addition to boscalid, I also wanted to assess the sensitivity of some *A. solani* isolates to the active ingredient fluopyram *in vitro*. In contrast to the boscalid sensitivity – with EC_{50} values of more than

20µg/ml – the sensitivity to fluopyram was below 5µg/ml for all tested isolates. The varying influence of different active ingredients with the same mode of action was also demonstrated by Rehfus et al. (2018) with *Sdh*-gene mutant isolates of *Zymoseptoria tritici* (*SdhB*-N225T, *SdhB*-T268I, *SdhC*-T79N, *SdhC*-W80S, *SdhC*-N86S, *SdhC*-H152R, *SdhC*-V166M). In previous studies a lack of cross-resistance between boscalid and fluopyram was already discussed for *Corynespora cassiicola*, *Podosphaera xanthii* or *A. solani* (Ishii et al. 2011; Gudmestad et al. 2013) and I can confirm this lack of cross-resistance with my data. However, to analyze the correlation for each of the mutant genotypes, a higher number of strains per mutation would be necessary. Although all calculated EC_{50} values for fluopyram were below 5µg/ml, a slight tendency for higher EC_{50} values of *SdhC*-H134Q mutant isolates (between 1 and 5µg/ml) compared to most of the other strains with different *Sdh*-genotypes (<1µg/ml) is visible. Bauske et al. (2018b) observed an increase in *SdhD*-D123E mutant isolates in the field after in-furrow applications of fluopyram, which clearly shows that fluopyram selects for specific *Sdh*-mutations. In the case of the *SdhC*-H134Q mutation, the slightly higher EC_{50} values indicate that this mutation might be less sensitive to fluopyram compared to the other *Sdh*-genotypes. Thus, if there´re still some *SdhC*-H134Q mutant isolates in the field, the application of fluopyram might lead to an increasing occurrence of this mutation. However, Bauske et al. (2018b) could only demonstrate an advantage of *SdhD*-D123E mutant strains in greenhouse and field trials, but not *in vitro*. This leads me to the assumption that the EC_{50} values might not be a sufficient indicator of fungicide efficacy and pathogen behavior in more natural environments. Still, they can help to categorize the single strains in a first approach. Nevertheless, in *planta* experiments are necessary to complete the picture.

To include the interaction with potato plants and to get closer to field conditions, the efficacy of 100µg/ml boscalid was evaluated in greenhouse trials in the next step. These kinds of *in planta* experiments can offer a higher throughput in the number of tested isolates and is not that time consuming as a field trial. Gudmestad et al. (2013) already observed a reduced sensitivity of isolates to 100µg/ml boscalid *in vivo*, which were of unknown genotype, but showed a relatively high EC_{50} value *in vitro*. In my greenhouse trials, nearly 100% fungicide efficacy was assessed for the *Sdh*-wild type isolates, and an impact of *Sdh*-gene mutations was clearly visible. Early blight was still controlled when isolates with *SdhB*-H278R and *SdhC*-H134Q were used for inoculation. In contrast, only little efficacy was assessed after inoculation with the frequently occurring *SdhB*-H278Y and *SdhC*-H134R mutant strains. These results may indicate that strains with a less effective mutation (*SdhB*-H278R and *SdhC*-H134Q) are outcompeted by more resistant and frequently occurring genotypes (*SdhB*-H278Y and *SdhC*-H134R). This could possibly explain their low frequencies and transient appearance and, in turn, the increasing numbers of isolates with either *SdhB*-H278Y or *SdhC*-H134R mutations, which show reduced boscalid sensitivity *in vivo*. This influence of different *Sdh*-gene mutations in *A. solani* on the efficacy of boscalid *in vivo* was also demonstrated by Bauske et al. (2018b). In contrast to my results, the authors didn´t observe significant differences between *Sdh*-wild type and *SdhC*-H134R mutant isolates at 100µg/ml boscalid, but at lower boscalid concentrations. The boscalid efficacies for the other tested mutant strains (*SdhB*-H278R, *SdhC*-H133R and *SdhD*-D123E) in the study of Bauske et al. (2018b) varied between 95 and 100% for a concentration of 100µg/ml, whereas I observed significantly lower efficacies for the *SdhB*-H278R mutant strains compared to the *Sdh*-wild type. Isolates with either *SdhC*-H133R or *SdhD*-D123E mutations were not included in my

study. One reason for these differences might be the use of different host plants (potato vs. tomato). This indicates that results from tomato might not always be directly transferrable to potato and that research with potato plants is essential to understand plant-pathogen-fungicide interaction in detail.

To get as close to natural conditions as possible, field trials with artificial inoculations were conducted to observe the interaction between plant, pathogen, environment, and fungicides between 2016 and 2018. In the field trials, I used three isolates for inoculation either with *Sdh*-wild type or two different *SdhC*-H134R mutants. The *SdhC*-H134R gene mutation was chosen because it´s the predominant mutation in Germany. In addition to the fungicide Cantus®, which obtains boscalid as active ingredient, three other SDHI fungicides – with varying active substances – were included. The use of infected barley grains as artificial inoculum source has proven to be very efficient. With these kernels, a specific inoculation with single *A. solani* strains is possible and enables to generate *Sdh*-wild type or *Sdh*-mutant predominance in the field. Moreover, early blight infection is supported, even if the weather conditions are not favorable for the pathogen. Natural infection with *A. solani* starts with the soil-borne inoculum, and first leaves near the soil are infected. During the season, early blight develops from the lower leaf levels to the upper leaf levels. This infection process is promoted by the infected kernels lying on the soil between the rows. In general, artificial inoculation leads to higher disease pressure, and this can promote the diversification in fungicide performance. However, this also limits the comparability of field trials with fields in practice. Nevertheless, the interactions of plants, pathogens, and the environment are essential to get a clear picture of how SDHI-fungicides performance is affected by the presence of particular *Sdh*-gene mutated isolates. The efficacy of Cantus® was highly reduced when the field was inoculated with *SdhC*-H134R mutant isolates in 2016 and 2017 compared to the *Sdh*-wild type inoculation. For the *SdhC*-H134R mutant 2, a complete loss of efficacy was assessed in all three years. This difference in effectiveness for the two *SdhC*-H134R mutant isolates shows a high influence of single strains, partially independent from *Sdh*-genotypes. These behaviors might be explainable by additional differences in other parts of the genome (R. Stam, personal communication) that may have an impact on fungicide sensitivity or fitness of the single isolates. In contrast, even under increasing disease pressure after inoculation with the *Sdh*-wild type isolate, Cantus® proved potent in the control of early blight in 2016 and 2017. In 2018, the rAUDPC of the untreated, but inoculated controls were as high as for the natural infection. This could have two possible reasons: either the artificial inoculation was not successful at all, or the natural disease pressure was extremely high in this year so that the artificial inoculum was not able to increase the pathogen density further. The very early and rapid disease progression in the untreated controls suggests a very high disease pressure in June and July in 2018. In my weather data analysis, I counted many more days with favorable conditions for early blight infection during these two months in 2018 compared to 2016 and 2017. Therefore, I needed to exclude this year because a predominance of our inoculated strains over the natural inoculum was not ensured. However, for the years 2016 and 2017, I observed successful artificial inoculation. As it was already shown *in vitro*, the active ingredient can influence the efficacy of an SDHI-fungicide. For the *Sdh*-wild type inoculation, all three additional SDHI-fungicides reduced rAUDPC values in 2016 and two of three did so also in 2017. The controlling effect of the tested SDHIs was significantly different among each other. Hence, even without a mutation in

the *Sdh*-gene, the impact of active ingredients and probably also their formulation, is clearly visible. For the *SdhC*-H134R mutant 1 inoculation, a significantly reduced rAUDPC compared to the untreated control was observed for two of three SDHIs in 2016 and only for one of them in 2017. A comparable picture was obtained for the second *SdhC*-H134R mutant strain – two out of three additional fungicides reduced the rAUDPC significantly compared to the respective control in both 2016 and 2017. In 2018, similar results were achieved, but due to the possibly insufficient artificial inoculation, they´re not discussed in detail. Overall, the efficacy of the tested SDHI-fungicides was negatively influenced by the predominance of *Sdh*-mutated strains. However, the active ingredient can significantly influence the efficacy in the field with and without predominance of *Sdh*-mutated strains. Therefore, not only alternation between fungicides with different mode of actions (SDHIs, QoIs, DMIs) is important, but also varying products from the same fungicide group with different active ingredients can be part of a sustainable resistance management. Nevertheless, improved fungicide resistance management might be able to slow down resistance development, but it likely won´t stop it completely. Therefore, it is also essential to know more about the impact of the *Sdh*-mutations on the pathogen itself. In some cases, it has been shown that mutations, which lead to less fungicide sensitivity, can in turn have a negative impact on the fitness of these isolates (Hawkins and Fraaije 2018). In some studies a ban or reduction of the use of specific substances lead to a reversion of the resistance (van Leeuwen et al. 1979; Kublin et al. 2003). If *Sdh*-mutations in *A. solani* would also have a noticeable negative impact on the fitness, we might have the opportunity to reverse resistance in the field. Based on that, I also wanted to have a closer look into the fitness of *Sdh*-mutated *A. solani* isolates compared to *Sdh*-wild type isolates.

4.2.1 *The fitness of A. solani isolates with mutations in Sdh-genes*

Fungicide resistance is often associated with possible fitness costs, which may result in an evolutionary trade-off. For several different fungicide groups (e.g. DMIs, QoIs, SDHIs) mutations in the target region of various fungi are observed to negatively influence the fitness of the mutated strains compared to the respective wild type-genotype (Hawkins and Fraaije 2018). If mutated isolates proved to have a disadvantage in comparison to the respective wild type strains, a stop of the application of the respective fungicide may, over time, lead to a predominance of wild type strains in the fields again. This strategy led to a reversion in resistance of *Salmonella* spp. to tetracycline (van Leeuwen et al. 1979) or *Plasmodium falciparum* to chloroquine (Kublin et al. 2003). Although these examples are not from the fungicide field, it could also work for fungicide-resistant pathogens. Allen et al. (2017) also stated that there´re several different ways to reverse resistance – depending on the population biological processes that can drive reversion– but in a first step, we need more profound knowledge about the impact of *Sdh*-mutations on *A. solani*. In recent years, Landschoot et al. (2017a) and Bauske and Gudmestad (2018) assessed the *A. solani* spore germination, mycelial growth and disease severity *in vivo* (only Bauske and Gudmestad (2018) included *in vivo* trials) of *Sdh*-mutated and *Sdh*-wild type *A. solani* isolates and couldn´t find any disadvantages for mutated strains. When we look into other pathosystems, similar results were observed for *A. alternata* on peach fruits or pistachio (Avenot and Michailides 2007; Fan et al. 2015). In contrast, Amiri et al. (2014) demonstrated not only differences between *Sdh*-wild type and *Sdh*-mutated *B. cinerea* isolates, but also the influence of testing growth media. They couldn´t find any

difference in sclerotia production of intermediate medium, but was able to see differences on PDA medium. These findings clearly show the impact of the testing media on the results and need to be considered for further fitness tests. To complement our studies on SDHI resistance, I also included the assessment of several fitness parameters: spore production capability, spore germination, mycelial growth, disease development in greenhouse, and toxin production. For some of these parameters, I measured at two different time points, to get more knowledge about the development of the single strains over time. This approach isn´t part of previous studies with *A. solani* from Landschoot et al. (2017a) and Bauske and Gudmestad (2018). Regarding the spore production capacity in our tests, no significant differences were observed between the groups after two and three weeks. However, the spore density of the SdhC-H134R mutant isolates was by trend higher after three weeks compared to the Sdh-wild type isolates, whereas no trend was visible after two weeks. This indicates that an extended incubation period may lead to significant differences, which are not visible after two or three weeks. For the spore germination, no differences were observed between the groups from two or three weeks old plates.

In contrast to Landschoot et al. (2017a) and Bauske and Gudmestad (2018), we found significantly faster mycelial growth of both *SdhB*-H278Y and *SdhC*-H134R mutant isolates compared to the *Sdh*-wild type after a growing period of two weeks on V8 agar. After one week, significant differences were only observed between the mutant groups. This inconsistency between week one and week two supports again my assumption, that for some parameters the incubation time needs to be extended in order to get reliable data. Furthermore, I used a different kind of growth media, compared to the other studies and Amiri et al. (2014) already demonstrated the possible impact of the testing media on the results. This faster mycelial growth may speed up soil colonization of the mutated isolates compared to the wild types. However, this possibility needs to be confirmed with specific soil colonization tests, e.g. soil inoculation and subsequent determination of fungal DNA. Presumably, one of the most relevant parameters is the disease development on the leaves. Therefore, I conducted a greenhouse trial, to determine the aggressiveness of the different genotype groups. I observed a significantly higher disease severity caused by the *Sdh*-wild type isolates compared to both *Sdh*-mutant groups. Moreover, no difference could be detected between the individual *Sdh*-mutations. This result indicates a possible advantage of the *Sdh*-wild type isolates in comparison to the mutated strains. The outcome of this experiment should not be overestimated, because the trial was only conducted once. The last parameter that I included in my studies – and wasn´t considered in previous studies in context with fitness – was the toxin production in liquid culture. Malandrakis et al. (2013) reported that laboratory *Aspergillus carbonarius* strains, which are resistant to phenylpyrrole, show reduced ochratoxin production *in vitro* and *in vivo* on grapes. In a study of D'Mello et al. (2001) Carbendazim-resistant *Fusarium sporotrichoides* strains produced higher quantities of T-2 toxin, but also less Neosolaniol compared to wild type strains. Both D'Mello et al. (2001) and Malandrakis et al. (2013) didn´t check for the genetic background of their tested isolates, so it remains unclear whether their isolates show one or several mutations. This circumstance makes it difficult to compare their results with mine. However, they demonstrate the general possibility that fungicide resistant strains may differ in their toxin production ability. *A. solani* is known to be able to produce several different toxins (Andersen et al. 2008; Ostry 2008;

Pinto and Patriarca 2017) and in my isolates, I detected Alternariol (AOH) and Alternariol methylether (AME). I couldn´t find any significant differences, but the *Sdh*-wild type group produced by trend higher amounts of both AOH and AME. It is likely that my isolates produce also other toxins. Especially the choice of the media, incubation time and cultivation conditions can highly influence toxin production. Whereas Andersen et al. (2008) extracted the toxins AOH, Altersolanol A, Altertoxin and Macroscopin from dichloran–Rose Bengal–yeast extract–sucrose agar plates (Frisvad 1983), which were cultivated for 14 days, Cotty and Misaghi (1984) used casamino acids-enriched liquid media (White and Starratt 1967) for 35 days and found Zinniol. Similar to my results, Lee and Yu (1995) found only AOH and AME in rice cultures inoculated with *A. solani*. Although there´s no clear difference between the genotype groups, the production of both AOH and AME was strongly positively correlated with each other in the *SdhB*-H278Y mutant group. In contrast, no correlation was found in the *Sdh*-wild type and *SdhC*-H134R group. Markoglou et al. (2011) demonstrated that highly Anilinopyrimidine-resistant *Aspergillus parasiticus* strains produced higher amounts of Aflatoxins than the wild type strain, but the authors also observed less Aflatoxin production in moderately resistant *A. parasiticus* strains compared to the wild type. In the study of Markoglou et al. (2011), they didn´t analyze the genetic background of the isolates, so we don´t know whether the single isolates possess only one mutation, or several different ones. However, this may indicate that isolates with different resistant-levels could also show different toxin production abilities. Perhaps the different *Sdh*-mutations in *A. solani* also lead to varying toxin production abilities, but to confirm this, further tests are needed. Nevertheless, the observation of a by trend lower total amount of AOH and AME in both mutant groups compared to the *Sdh*-wild type also supports the possibility of an impact of the *Sdh*-mutations on the toxin production. To confirm these findings, further research is needed – especially a repeated measurement of the already included isolates and testing of many more isolates.

To sum up the fitness tests, for the parameters spore production and spore germination, I couldn´t observe significant differences between the genotypes. A significantly faster mycelial growth was assessed for both mutant groups compared to the *Sdh*-wild type only at the second date of assessment. This shift from not significant at the first date of assessment to significant at the second date supports that longer incubation times might support assessment of small quantitative differences in phenotypes. In contrast to the three described *in vitro* parameters, the disease development *in vivo* shows a significant advantage for the *Sdh*-wild type isolates over the respective mutants. These observations are accompanied by the by trend higher toxin production of AOH and AME in liquid culture for the *Sdh*-wild types compared to the *Sdh*-mutants. Overall, the *Sdh*-mutations seem to have an effect on the fitness, but this needs to be analyzed further. The correct answering of the question, if there´re noticeable fitness costs or not, can strongly affect the way, how early blight control and the fungicide resistance development should be managed. In case of ongoing consecutive use of SDHI-fungicides, the selection pressure would be very high and will lead to a predominance of *Sdh*-mutated isolates in the fields. If *Sdh*-mutated isolates proved to have a disadvantage in comparison to the *Sdh*-wild type strains, a stop of SDHI fungicide applications would, over time, lead to a predominance of *Sdh*-wild type strains on the fields again.

4.3 The potential of Trichoderma spp. as BCAs

There is no doubt that the development of fungicide resistance of *A. solani* will pose a challenge for future potato production. Therefore, it is essential to think about ways to slow down the selection of mutated isolates and to control early blight by alternative measures. On top, in recent years, the public pressure on farmers is continuously rising, because the use of chemical plant protection products is associated with negative consequences for the environment. In this study, I thus assessed the potential for *Trichoderma* as BCA against *A. solani* on potato on three different scales: *in vitro*, *in vivo,* and in the field. The use of BCAs should not be seen as a strict alternative to chemical plant protection, but as a further pillar of integrated pest management and as a possibility to slow down resistance development against chemical active ingredients.

In *in vitro* dual culture tests, the ability of *Trichoderma* to inhibit growth of *A. solani* was assessed. All strains showed an inhibitory effect on growth of *A. solani*, but two *T. atroviride* isolates performed best with around 65% reduced mycelial growth compared to the control plates. In studies with *T. harzianum* and *A. solani* from tomato, Lakhdari et al. (2018) and Mazrou et al. (2020) also showed growth inhibition effects of around 60%. Interestingly, the two isolates belonging to *T. harzianum* in my study showed significantly different growth inhibition effects (35 and 50%). Consolo et al. (2012) and El Gamal et al. (2018) recorded similar data. They also reported a wide range of growth reduction in dual culture tests with different *T. harzianum* isolates and *A. solani* isolates from tomato and potato, respectively. The production of volatile compounds is often discussed as one possible mechanism, which contributes to the inhibition of other fungi. Amin et al. (2010) showed the ability of some *Trichoderma* derived volatile compounds to reduce mycelial growth of seven plant pathogenic fungi, e.g. *A. brassicicola*, *R. solani* or *F. oxysporum*. Moya et al. (2018) also showed different inhibitory effects of VOCs emitted by different *T. harzianum* strains on mycelial growth of *P. teres in vitro*. The study of Moya et al. (2018) clearly stated how important it is – even in the context of volatile compounds –, to test several strains from the same species. However, it is likely that volatile metabolites may also contribute to the inhibitory effect in my dual culture tests. In the case of dual culture tests, the ability of *Trichoderma* to compete with *A. solani* about nutrients and space is one of the predominant mechanisms. Especially the rapid growth of *Trichoderma* compared to *A. solani* was apparently a big advantage in the dual culture tests. This rapid growth of *Trichoderma* was the reason, why I decided to place *A. solani* two days earlier on the plates to give *A. solani* the chance to establish themself. Several studies showed the ability of *Trichoderma* to produce antifungal secondary metabolites (Reino et al. 2007; Vinale et al. 2014), so perhaps also the timing of the confrontation on the agar plates may have an effect on the results. If *Trichoderma* is placed on the plates on the same day, or even two days earlier then *A. solani*, the inhibitory effect may have been even stronger. At least for volatile compounds, Lee et al. (2015) observed an impact of *Trichoderma* culture age on the compound composition. Although we can´t clearly state which mechanisms are responsible for the inhibition, the *in vitro* dual culture tests can help to screen for promising *Trichoderma* strains. However, to point out clearly all mechanisms involved in this inhibition, further research is needed.

In the next step, I tested the six *Trichoderma* isolates in greenhouse trials as foliar application of spore suspensions. All tested strains resulted in a reduction of disease severity. This reduction ranged

from 15 to 57%. As Al-Hazmi and TariqJaveed (2016) showed, rising spore concentrations of *T. viride* in soil lead to better control of the root knot nematode *Meloidogyne javanica* on tomato. A higher spore density probably could have also increased the efficacy in my greenhouse trials. Most published studies with *A. solani* and *Trichoderma* are conducted with soil treatments in tomato plants. Independent from different application methods, Fontenelle et al. (2011) also observed a broad range of disease reduction from 31 to 95% for different *Trichoderma* strains in soil. This high variation between the single strains underlines how important it is to look not only into one *Trichoderma* species, but to consider different species and several strains from the same species. In contrast to the dual culture tests, the *T. atroviride* isolates showed medium disease control with no significant differences to other isolates in the greenhouse. The *T. harzianum* isolate 20761 had the strongest disease-suppressive effect. The significant difference between the isolates *T. harzianum* 20761 and *T. harzianum* 20770, which was observed *in vitro*, was also visible *in vivo*. Unlike to the performance in the dual culture tests, I saw by trend a better disease control by *T. asperellum* when compared to the other tested isolates, except *T. harzianum* 20761. The *T. asperellum* 20866 isolate was initially gained from parsley (Körmöczi et al. 2013), which belongs to the family of *Apiaceae*. This demonstrates the diversity of *Trichoderma* and that it´s worth looking into isolates from other plant families to find new BCAs. Regarding the question, which mechanisms lie behind the controlling effect of *A. solani* in greenhouse, several answers are possible. Several researchers have demonstrated that *Trichoderma* isolates can survive on leaf, flower or berry surface after foliar treatment for some time (Elad 1994; Dodd et al. 2004; Freeman et al. 2004; Longa et al. 2008). This leads to the suggestion that *Trichoderma* can directly interact with the foliar pathogen when it lands on the surface. There might be some fungal products already present in the applied spore suspension of *Trichoderma*, which support the inhibition of *A. solani*. Interaction with the pathogen was shown for exochitinases derived from *Trichoderma* (Brunner et al. 2003). These enzymes initiate cell wall degradation of the host fungus, and released chitin fragments in turn lead to the expression of more CWDEs in *Trichoderma* (Brunner et al. 2003). This process can also be initiated from the spores themselves, because it was shown by Brunner et al. (2003) that a small amount of exochitinases is constantly released from *Trichoderma* to sense other fungi.

Another possible explanation would be that the treatment with *Trichoderma* induced host defense responses. Induced resistance could either be triggered by the spores themselves, or by secondary metabolites, which were already produced before harvesting the spores or directly on the leaf surface. Chowdappa et al. (2013) showed increased levels of defense-related enzymes (e.g., peroxidase or polyphenol oxidase) in tomato after seedling treatment with *T. harzianum*. The ability of *Trichoderma* derived metabolites and proteins to induce resistance was proven for several compounds like 6-pentyl-α-pyrone, avirulence-like (Avr) proteins or CWDEs (Harman et al. 2004; Mazzei et al. 2016). Hence, the direct contact between the *Trichoderma* spores, including their secondary metabolites and secreted proteins, and the plant surface, may enhance plant defense mechanisms and support the control of *A. solani*. The *Trichoderma* treatment was carried out 24h before the inoculation with *A. solani*. Yedidia et al. (1999) demonstrated an upregulation of peroxidase activity in leaves 24h after seedling treatment with *Trichoderma*, whereas the peak of chitinase and peroxidase activity in leaves was observed after 72h. For chitinase, Grover (2012)

named several studies where the role of this protein in defense response of different host plants against pathogens is well described. Similar to chitinase, peroxidase activity also plays an important role in plant defense response to pathogens (Choi et al. 2008; Daudi et al. 2012; Mammarella et al. 2015). Perhaps the early blight controlling effect would have been even stronger if the inoculation of *A. solani* would have been taken place later in my experiments.

For now it remains unclear which mechanism is responsible for less effective infection of *A. solani*, but it is likely, that a mixture of mechanisms explains the reduced disease severity after *Trichoderma* treatments in the greenhouse trials. Therefore, further research is needed to identify active *Trichoderma* compounds and their mode of actions.

To complement the studies with *Trichoderma*, field trials in four consecutive years were conducted. To evaluate the *Trichoderma* treatments, the rAUDPC and the efficacy at the time point with around 20% disease severity in the untreated control were analyzed. Regarding the rAUDPC, a significant reduction was only observed in 2016 with three *Trichoderma* strains and the fungicide control. In the other three years, the only significant impact on the disease severity was measurable with the fungicide treatment in 2018. These differences between the years are not very surprising, because in field trials the impact and interaction with environmental factors can be very high. Perelló et al. (2009) conducted two field trials in consecutive years with *Trichoderma* as BCA against *M. graminicola* in wheat and they also reported good efficacy of the treatments in the first year and no effect in the second year. They included two different wheat cultivars in their trials and they could clearly show that the cultivar played an important role in the outcome of the *Trichoderma* treatments. These results may raise the question, if the potato cultivar has had an effect on the efficacy in our trials as well, because in 2016 and 2018 the cultivar Lady Amarilla and in 2017 and 2019 Maxilla was used. Beside Perelló et al. (2009), other studies also observed an influence of the cultivar on plant responses to Trichoderma (Mutawila et al. 2011; Duc et al. 2017). However, I could only observe a significant reduction in one of the two years with the same cultivar, so there might be an impact of the cultivar, but there must be other reasons as well.

To get a more detailed picture, I analyzed the efficacy of the treatments at one specific time point, namely when the disease severity in the untreated control reached around 20%. This level of disease severity was chosen on the one hand because it indicates the start of the progression-phase and on the other hand to evaluate the performance of the treatments at a moderate disease pressure. We observed partial high variances within the repetitions of one treatment and between years. Overall, early blight reduction was observed in the years 2016, 2017, and 2019, respectively, in consequence of the *Trichoderma* application. While the efficacy was around 50 and 25% for many of the tested *Trichoderma* treatments in 2016 and 2017, respectively, a disease reduction of about 10% was determined for most of the treatments in 2019. These efficacies have to be seen in the context of the fungicide efficacy, which was 60% in 2016, 50% in 2017, and only 40% in 2019. In 2018, nearly no controlling effect of any treatment (including the fungicide) was assessed. These results indicate that there´s probably no impact of the cultivars in this stage. As already discussed for the fungicide trials, the disease pressure in 2018 was very high, and there was a very early and rapid disease development, which could explain the low efficacy of all control measures in this particular year. The

treatments with the commercial BCA products were, in most cases, not as efficient in controlling early blight as my own spore solutions, which perhaps indicates an advantage for freshly harvested spores. However, we have to keep in mind that the commercial products I included in my trial as foliar applications are initially registered for soil treatments. A positive synergistic effect of the mixture of three different *Trichoderma* strains was not visible. However, this treatment was only included once in 2017 and a second time in 2018, but in 2018 nearly no treatment did show a controlling effect. Besides the very high disease pressure in 2018, another possible reason for the lower or missing control of early blight in the years 2018 and 2019, respectively, compared to 2016 and 2017, could be the timing of the applications. In the first two years, two *Trichoderma* treatments were done before the first symptoms appeared. In the following two years, only one or no application, respectively, were conducted before the first early blight infection occurred. These circumstances might have led to an insufficient or missing activation of defense response mechanisms, e.g., the upregulation of chitinase and peroxidase activity or accumulation of γ-aminobutyric acid (Yedidia et al. 1999; Mazzei et al. 2016). However, especially the year 2017 should not be overestimated because the disease severity was quite low, and the overall conditions of the plants were not as good as in the other years. This was due to the late planting and unfavorable soil and weather situation in that season. After the first treatment, three additional leaf applications (four in 2019) followed in a spray-interval of mostly 7 to 10 days. For a couple of treatments, the circumstances –rainfalls or heavy winds –allowed to spray in a 14-day interval only. Some studies showed a survival of *Trichoderma* strains on leaves for at least seven days, but with a rapid decrease in cell forming units after two to three days (Freeman et al. 2004; Longa et al. 2008). One can assume that *Trichoderma* strains were vivid for a certain period on the leaves, but the efficacy decreased after the single treatments. To increase the disease-controlling effect of *Trichoderma*, application on leaves in short time intervals may be beneficial. Nevertheless, we also have to keep in mind that the soil was artificially inoculated with *A. solani* at the beginning of the season. Hence, the disease pressure on the field was higher when compared to natural infection. McLean et al. (2012) demonstrated a reduced control of onion white rot by *T. atroviride* under higher disease pressure on the field compared to low or medium disease severity. Under natural infection conditions, the supportive effect of *Trichoderma* might have been even stronger than in my study.

In contrast to the *in vitro* and *in vivo* trials, no significant differences in efficacy were observed between the single strains in field trials at 20% disease severity. Regarding the rAUDPC values, the *T. harzianum* strain 20761 led – together with two others – to a significant reduction compared to the untreated control in the field in 2016 and the same strain also performed best in the greenhouse trials. The other two isolates – *T. atroviride* 20780 and *T. atroviride* 20781 –, which also significantly reduced the rAUDPC values in 2016, did not differ in comparison to other strains in the greenhouse trials. Hence, it´s difficult to judge whether the results from the field trials are comparable to the *in vitro* and *in vivo* trials. One big difference between greenhouse and field was the application technic. In the greenhouse trials, the whole plant was sprayed with a spore solution. In the field trials, the spraying was conducted with a backpack sprayer on the upper parts of the plants. This technical difference needs to be considered when we try to compare greenhouse trials with field trials. The

Trichoderma spores lie mostly on top of the plants, whereas the disease development starts from the ground (infected kernels and natural disease development). This circumstance likely reduces the ability of *Trichoderma* to serve as a mycoparasite – at least at the beginning of the disease, because direct contact between pathogen and BCA is limited. The mechanism, which may reduce disease severity at this stage, is probably the induction of resistance, as discussed for the greenhouse trials. These differences in the application method of *Trichoderma* may partially explain the lack of transferability, especially between greenhouse and field trials. Moreover, *Trichoderma* is more directly exposed to the environment under field conditions. This includes high temperatures, the exposure to UV-light during the day or rainfalls. These environmental conditions might also have contributed to the partial high variance within each treatment. During the field trials, we avoided to spray when rain was forecasted, but sometimes it was unpreventable. Hence, it´s possible that rainfalls in the days after spraying, washed off parts or all of the spore solution from the leaves. To enhance rain fastness of potential BCAs, several studies were conducted, e.g., with starch- and flour-based formulations, potassium lignate, and vegetable oil-based waterborne polyurethanes (McGuire et al. 1996; Tamez-Guerra et al. 2000; Zheng et al. 2020). In a follow-up project, it would be necessary to test new formulations for *Trichoderma*, in order to possibly enhance efficacy and persistency. Besides rain, heat and varying temperatures can also influence the effectiveness of *Trichoderma* treatments. Studies with *T. atroviride* demonstrated the highest growth rate at 30°C on PDA plates compared to lower temperatures (Longa et al. 2008). Over 37°C, no growth was observed. The conditions on media are, of course, better for fungal growth than a leaf surface, but the experiment at least shows the ability of *Trichoderma* to grow very well up to 30°C. Even though the applications of *Trichoderma* were conducted in the mornings, rising temperatures during the day might also inhibit or reduce activity on leaf surfaces. Unfortunately, it was not investigated by Longa et al. (2008), if the strains would be able to recover from the heat exposure under cooler conditions. In addition to high temperatures, the *Trichoderma* spores are also exposed to UV light after foliar treatment. In a pretest, I investigated *Trichoderma* growth on two different growing media and light conditions. The six tested *Trichoderma* strains were able to grow under UV-light. Hence, the exposure to sunlight on the plant surface should not inhibit *Trichoderma* activity. However, it might lead to some kind of changes in *Trichoderma* physiology because the phenotypic growth under UV-light was partially different compared to normal light (Fig. S 1). It could be possible, that the exposure to UV-light somehow stresses the soil fungus *Trichoderma*. This additional stress could probably lead to the production of more or other secondary metabolites, which could in turn increase or decrease the potential to act as BCA. In this context, the modulation of *Trichoderma* cellulase production in response to light was shown by Schmoll et al. (2005). In several other studies, the possible impact of abiotic stress factors, e.g. temperature, water activity or salt, has been shown on the metabolism of diverse fungi (Leong et al. 2006; Ochiai et al. 2007; Stoll et al. 2013; Medina et al. 2015).

Taken together, the exposure to varying environmental conditions on the leaf surface probably have influenced the efficacy of *Trichoderma* and increased variances within each treatment. Especially the research into adapted and more stable isolates together with new spore formulations might help to reduce this issue for the future. However, another opportunity to minimize the effect of abiotic

stress factors is the application of *Trichoderma* in soil. Longa et al. (2008) showed a higher survival rate of *Trichoderma* after soil treatments. Fontenelle et al. (2011) demonstrated good results for *Trichoderma* applied in soil to reduce *A. solani* infection on tomato. For potatoes, a seed tuber or in-furrow treatment with *Trichoderma* would also be an option, which needs to be investigated further. For controlling *M. graminicola* on wheat, Perelló et al. (2009) compared seed-coating and foliar applications with Trichoderma strains in the field and they could show that seed-coating is in most cases more efficient than foliar treatments, especially in an earlier stage of disease development. In this context, it would be very interesting to combine both methods in further field trials with potatoes. It would also be interesting to search for *Trichoderma* strains, which can parasite the soil-borne inoculum of *A. solani* during the colder season. Köhl and Schlösser (1989) analyzed several isolates of *Trichoderma* spp. for their cold tolerance and found some strains, which were able to parasite Sclerotia of *B. cinerea* at 5°C *in vitro*. This ability should also be included in further research to reduce early blight of potato sustainably.

In summary, these studies show the potential of *Trichoderma* as a BCA for *A. solani* in potato. It was also demonstrated that each *Trichoderma* strain can have different inhibitory potential and that we should use this source of diversity to find optimal isolates for continuing research. Beside further investigations in foliar treatments, the possibility to use *Trichoderma* as soil treatment should also be part of new research projects.

5. Summary/Zusammenfassung

Summary

The main aims of this thesis were first to investigate the spreading of *Sdh*-mutated *A. solani* isolates in Germany and the possible impact of *Sdh*-mutations on SDHI-fungicide sensitivity and general fitness of mutated isolates. Second, I wanted to analyze the impact of climatic changes – rising temperatures and less rain – on early blight disease development and third I wanted to evaluate the potential of *Trichoderma* as possible BCA to contribute to a sustainable control of early blight in the future.

Climate change is currently one of the most important challenges to humankind. The consequences are not yet fully assessable, but more and longer periods of heat and drought are predicted for European countries as it was already the case in recent years (Olesen et al. 2011). Hence, one of my goals was to assess the possible impact of rising temperatures on early blight infection. Data demonstrate that higher temperatures can increase disease severity, and the reasons for this need to be further analyzed. Besides temperature, the availability of free water on the leaves may be crucial for a successful infection of potato by *A. solani*. In greenhouse trials, I found a minimum leaf wetness duration of 8h to be sufficient for successful infection by *A. solani*. The analysis of the weather data from 2016-2019 shows that there are still many days with at least 8h of leaf moisture in each year, especially because of dew during the night. In the framework of these studies, I also found out that *A. solani* strains from diverse alternative hosts and organs – namely potato leaves, potato tuber, tomato (*Solanum lycopersicum*), wild tomato, black nightshade *(Solanum nigrum*) and thistle (*Cirsium arvense*) – can infect potato plants in greenhouse trials. Hence, even if there´s no potato on the field in one year, weeds may support the reproduction of inoculum. I also tested the isolates from different hosts for their spore production and germination rate and found a negative correlation between spore production and germination rate. This raises questions on a potential trade-off between those fungal growth characteristics and should be analyzed in more detail. Based on the predicted climatic conditions, which seem to be favorable for *A. solani*, the future control of early blight is likely to be increasingly challenging. Succinate dehydrogenase inhibitors (SDHIs) are among the most frequently used specific single site fungicides that control *A. solani.* The accumulation of isolates with target site mutations in *Sdh*-genes is often the consequence of the extended use of fungicides with a single-site mode of action. In a general monitoring, I assessed the distribution of *Sdh*-mutated isolates over Germany between 2013 and 2016. In general, the amount of isolates with *Sdh*-mutations constantly increased over time with *SdhB*-H278Y and *SdhC*-H134R being the most frequently occurring mutations. In addition to these two mutations, the *SdhB*-H278R and *SdhD*-D123E mutations occurred in lower amounts. Moreover, B. Adolf, a member of our working group at the chair of phytopathology, detected a new target site mutation: *SdhC*-H134Q. To get a clearer picture of the impact of the individual *Sdh*-mutations on the disease control with SDHIs, I conducted *in vitro*, greenhouse trials, and field experiments. Most of the tests were performed with one of the most frequently used active ingredients to control early blight: boscalid. The presence of *Sdh*-mutations was associated with increased EC_{50}-values *in vitro* and decreased fungicide efficacy *in vivo* and in the field. I also tested the alternative SDHI fluopyram, and the observed EC_{50} values were much lower when compared to the ones for boscalid. Moreover, I included some additional

fungicides with different active SDHI ingredients in the field trials. Overall, the efficacy of the tested SDHI-fungicides was negatively influenced by the use of *Sdh*-mutated strains as inoculum. However, the kind of active ingredient significantly influenced the efficacy of disease control in the field with and without inoculation with *Sdh*-mutated strains. Therefore, alternation of products from the same fungicide group but with different active ingredients should be considered for future fungicide resistance management. To complement my studies with SDHI resistance, I also included the assessment of several fitness parameters: spore production capability, spore germination, mycelial growth, disease development in greenhouse, and toxin production. For the parameters spore production and spore germination, no significant difference was observed between diverse *Sdh*-genotypes. A significantly faster mycelial growth was measured for both *SdhB*-H278Y and *SdhC*-H134R mutants compared to the *Sdh*-wild type after an extended incubation time. In contrast, the disease development *in vivo* shows a significant advantage in the development of disease symptoms for the *Sdh*-wild type isolates. These observations can be possibly explained by the by trend higher toxin production of AOH and AME in liquid culture from the *Sdh*-wild type strains compared to the *Sdh*-mutants. Overall, the *Sdh*-mutations seem to have a potential impact on the fitness, but this needs to be analyzed in more detail and with more isolates. It appears obvious that the fungicide resistance development of *A. solani* will pose a challenge for future potato production. Therefore, it is very important to think about alternative ways to control early blight and to reduce pathogen population size for slowing down the spreading of mutated isolates. To this end, I analyzed the potential of *Trichoderma* spp. as Biological Control Agent (BCA) against *A. solani* on potato on three different scales: *in vitro*, *in vivo,* and in the field. In the dual culture tests, six *Trichoderma* strains proved the ability to reduce *A. solani* growth on different levels. In greenhouse trials with foliar application, all *Trichoderma* isolates caused a reduction of disease severity again with varying efficacies between the single strains. In field trials, early blight reduction by *Trichoderma* was observed at the time point of a moderate disease pressure (around 20% in untreated control) in three out of four years, but the efficacies varied between each year and between the included isolates. On examination of the whole season, there was a significant reduction of the rAUDPC values in 2016 for with three of the tested *Trichoderma* isolates. Overall, these studies show the potential of *Trichoderma* spp. as a BCA against *A. solani* on potato. Apparently, each *Trichoderma* strain performs differently. Hence, the diversity of the *Trichoderma* genus may provide a future resource to find optimal isolates for continuing research and optimizing disease control. Taken together, the innovation of early blight control in potato will gain importance in order to guard yield in times of global warming and rapidly developing fungicide resistance. I hope that this work can provide support for potato crop protection and make it more sustainable in the future.

Zusammenfassung

Die Hauptziele dieser Arbeit waren erstens die Untersuchung der Verbreitung von *Sdh*-mutierten *A. solani* Isolaten in Deutschland und dessen möglicher Einfluss auf die SDHI-Fungizid Sensitivität und die allgemeine Fitness der Isolate. Zweitens wollte ich den Einfluss klimatischer Veränderungen – steigende Temperaturen und weniger Regen – auf die Entwicklung der Dürrfleckenkrankheit analysieren und drittens wollte ich das Potenzial von *Trichoderma* als möglicher BCA (Biological Control Agent) untersuchen, um die gezielte Kontrolle der Dürrfleckenkrankheit in Zukunft nachhaltiger zu gestalten.

Der Klimawandel ist derzeit eine der wichtigsten Herausforderungen für die Menschheit. Die Folgen sind noch nicht vollständig absehbar, aber es werden mehr und längere Hitzeperioden für europäische Länder vorhergesagt, wie dies bereits in den vergangenen Jahren der Fall war. Daher war eines meiner Ziele für diese Arbeit, die möglichen Auswirkungen steigender Temperaturen auf die Entwicklung der Dürrfleckenkrankheit zu untersuchen. Die Daten zeigen, dass höhere Temperaturen zu einem stärkeren Befall führen können und die Gründe dafür sollten weiter analysiert werden. Neben der Temperatur kann auch die Verfügbarkeit von Wasser auf den Blättern entscheidend für eine erfolgreiche Infektion mit *A. solani* an der Kartoffel sein. In Gewächshausversuchen konnte ich zeigen, dass für *A. solani* 8 Stunden Blattfeuchtigkeit ausreichend waren, um die Blätter erfolgreich zu infizieren. Die Analyse der Wetterdaten von 2016 bis 2019 zeigt, dass es in jedem der vier Jahre immer noch viele Tage mit mindestens 8 Stunden Blattfeuchtigkeit gab – vor allem auch bedingt durch den nächtlichen Tau. Im Rahmen dieser Arbeit konnte ich außerdem zeigen, dass *A. solani* Isolate, die von unterschiedlichen alternativen Wirtspflanzen und Organen isoliert wurden – Kartoffelblättern, Kartoffelknollen, Tomaten, Wildtomaten, Schwarzem Nachtschatten und Distel – ebenfalls die Kartoffelpflanze infizieren können. Das bedeutet, dass auftretende Unkräuter im Feld als Inokulumquelle dienen könnten und damit die Vermehrung des Inokulums fördern, auch wenn in einem Jahr keine Kartoffeln angebaut werden. Ich testete zudem die Isolate der einzelnen Wirtspflanzen auf deren Sporenproduktions- und Keimfähigkeit und konnte eine negative Korrelation zwischen Sporendichte und Keimrate feststellen. Anhand dieser Beobachtung stellt sich die Frage, ob es einen möglichen Trade-off zwischen diesen beiden Fitness-Parametern geben könnte, daher sollte dies weiter untersucht werden. Aufgrund der vorhergesagten klimatischen Veränderungen, die augenscheinlich von Vorteil für *A. solani* sein könnten, ist es sehr wahrscheinlich, dass die gezielte Kontrolle der Dürrfleckenkrankheit immer schwieriger werden wird. Die Succinat-Dehydrogenase-Inhibitoren (SDHIs) gehören zu den am häufigsten eingesetzten Spezial-Fungiziden gegen *A. solani*, die nur an einer bestimmten Stelle im Pilz wirken und somit ein erhöhtes Risiko für Resistenzentwicklungen aufweisen. Die Anreicherung von Isolaten, die eine Mutation in dem *Sdh*-Gen aufweisen, ist oft die Folge des übermäßigen Einsatzes von Fungiziden mit nur einem Wirkort. In einem deutschlandweiten Monitoring untersuchte ich die Verteilung von *Sdh*-mutierten *A. solani* Isolaten über einen Zeitraum von vier Jahren (2013 bis 2016). Im Allgemeinen stieg die Anzahl an *Sdh*-mutierten Isolaten stetig an, wobei die Mutationen *SdhB*-H278Y und die *SdhC*-H134R am häufigsten zu finden waren. Zusätzlich zu diesen beiden Mutationen, zeigten sich auch *SdhB*-H278R und *SdhD*-D123E Mutationen in geringer Menge. Außerdem konnte B. Adolf – eine wissenschaftliche Mitarbeiterin des Lehrstuhls für Phytopathologie – eine zu dem Zeitpunkt noch

nicht beschriebene Mutation nachweisen: *SdhC*-H134Q. Um einen besseren Überblick über den Einfluss solcher Mutationen auf die gezielte Kontrolle des Pathogens mit SDHI-Fungiziden zu bekommen, führte ich verschiedene in vitro, Gewächshaus- und Feldversuche durch. Ein Großteil der Versuche wurde mit einem der meistverwendeten Aktivsubstanzen durchgeführt, der für die Kontrolle der Dürrfleckenkrankheit eingesetzt wird: Boscalid. Das Vorhandensein von Sdh-Mutationen führte zu erhöhten EC_{50}-Werten in vitro und reduzierter Fungizid-Wirkung sowohl in Gewächshaus- als auch in Feldversuchen. Ich untersuchte auch die EC_{50}-Werte eines weiteren SDHI-Fungizids mit dem Wirkstoff Fluopyram und konnte feststellen, dass die EC_{50}-Werte, im Vergleich zu Boscalid, deutlich niedriger waren. Außerdem integrierte ich zusätzliche Fungizide mit unterschiedlichen SDHI Aktivsubstanzen in meine Feldversuche. Insgesamt zeigte sich, dass die Wirksamkeit der SDHI-Fungizide negativ durch das Einbringen von *Sdh*-mutierten *A. solani* Isolaten beeinflusst wurde. Dennoch hatten die eingesetzten Aktivsubstanzen einen signifikanten Einfluss auf die Wirksamkeit der Krankheitskontrolle, unabhängig davon, ob die Flächen mit *Sdh*-mutierten oder *Sdh*-Wildtyp Isolaten inokuliert wurden. Daher sollte eine Alternierung zwischen Produkten mit dem gleichen Wirkort (*Sdh*-Gen), aber mit unterschiedlichen Aktivsubstanzen ebenfalls beachtet werden, wenn es um ein zukünftiges Anti-Resistenzmanagement bei Fungiziden geht. Um meine Studien zu SDHI-Resistenzen zu vervollständigen, untersuchte ich auch einige Fitness-Parameter: Sporenproduktionsvermögen, Sporenkeimung, Myzelwachstum, Krankheitsverlauf im Gewächshaus und die Toxinproduktion. Für die Parameter Sporenproduktion und Sporenkeimung konnte ich keine signifikanten Unterschiede zwischen den einzelnen Sdh-Genotypen feststellen. Für das Myzelwachstum zeigte sich, nach einer verlängerten Inkubationszeit, ein signifikant schnelleres Wachstum sowohl für die *SdhB*-H278Y, als auch die *SdhC*-H134R Mutanten im Vergleich zu den *Sdh*-Wiltypen. Im Gegensatz dazu, zeigen die *Sdh*-Wildtyp Isolate einen signifikant schwereren Krankheitsverlauf im Gewächshaus als die *Sdh*-Mutanten. Diese Beobachtungen lassen sich möglicherweise durch die tendenziell höhere Produktion der Toxine AOH und AME in Flüssigkultur von *Sdh*-Wildtyp Isolaten im Vergleich zu *Sdh*-Mutanten erklären. Insgesamt scheinen die *Sdh*-Mutationen einen gewissen Einfluss auf die Fitness der Isolate zu haben, aber dies muss detaillierter und mit mehr Isolaten analysiert werden. Offensichtlich ist jedoch, dass die Fungizid Resistenzentwicklung von *A. solani* eine große Herausforderung für die zukünftige Kartoffelproduktion darstellen wird. Daher ist es sehr wichtig auch über alternative Möglichkeiten nachzudenken, um die Dürrfleckenkrankheit zu kontrollieren und die Populationsgröße von Pathogenen im Feld zu reduzieren, um die Ausbreitung von mutierten Isolaten zu verlangsamen. Zu diesem Zweck untersuchte ich das Potenzial von *Trichoderma* spp. als BCA gegen *A. solani* an der Kartoffel auf drei Ebenen: in vitro, in vivo und im Feld. In den so genannten „dual culture“ Tests führten die sechs *Trichoderma* Isolate zu einer Reduktion des Myzelwachstums von *A. solani* auf unterschiedlichem Niveau. Auch in den Gewächshausversuchen hatten alle sechs *Trichoderma* Isolate als Blattbehandlung eine Reduktion der Befallsstärke in unterschiedlichem Ausmaß zur Folge. Im Feld wiesen die Sprühapplikationen mit *Trichoderma* zum Zeitpunkt eines moderaten Befallsdrucks (ca. 20% in Kontrolle) in drei von vier Jahren eine gute Wirksamkeit gegenüber *A. solani* auf, die jedoch sowohl zwischen den Jahren als auch zwischen den Isolaten variierte. Bei der Betrachtung des Befallsverlaufs über die gesamte Saison hinweg, konnte eine signifikante Reduktion des rAUDPC-Wertes im Jahr 2016 bei drei der eingesetzten *Trichoderma* Stämme beobachtet werden. Insgesamt

zeigen diese Studien das Potenzial von *Trichoderma* als BCA gegen *A. solani* an der Kartoffel. Offenbar gibt es große Unterschiede zwischen den einzelnen *Trichoderma* Isolaten. Diese Diversität innerhalb des Genus *Trichoderma* kann dazu genutzt werden, die optimalen Isolate für die Kontrolle von Pflanzenkrankheiten und weiterführende Forschungsfragen zu finden. Alles in allem werden Innovationen im Bereich des *Alternaria* Managements in der Kartoffel weiter an Bedeutung zunehmen, um in Zeiten des Klimawandels und der rapiden Entwicklung von Fungizid Resistenzen, den Ertrag ausreichend zu schützen. Ich hoffe, dass diese Arbeit einen Beitrag dazu leisten kann, den Pflanzenschutz in der Kartoffel zu unterstützen und ihn in Zukunft nachhaltiger zu gestalten.

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7. Supplements

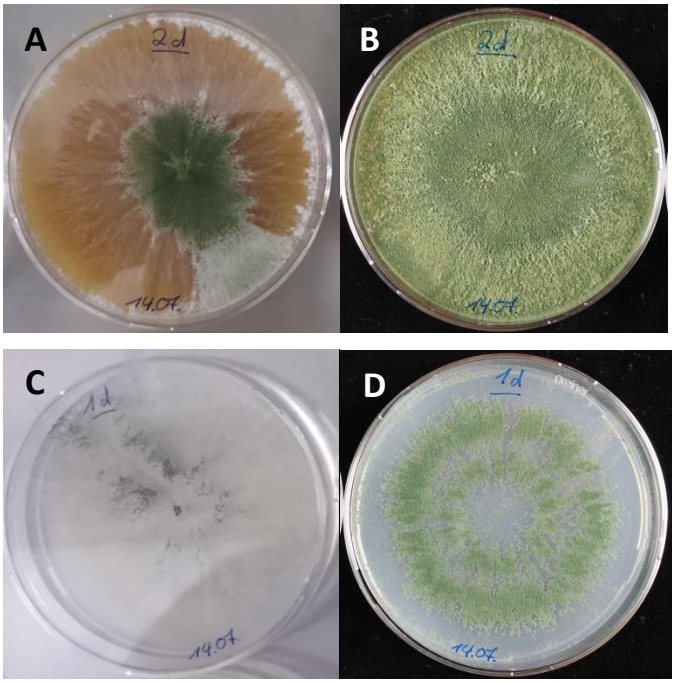

Figure S 1 Pictures from 10 day old Trichoderma plates from a pretest to get additional information about most suitable growing conditions for dual culture tests. (A) 12h UV-light/12h darkness on V8 agar; (B) At the window under normal day/night conditions on V8 agar; (C) 12h UV-light/12h darkness on SNA; (D) At the window under normal day/night conditions on SNA.

Abbreviations

AME	Alternariol monomethylether
AOH	Alternariol
BCA	Biological control agent
Dai	Days after inoculation
DMI	Demethylation inhibitor
EC_{50}	Effective concentration of an ingredient, that leads to 50% inhibition of an organism
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
PCR	Polymerase chain reaction
QoI	Quinone outside inhibitor
RH	Relative humidity
*Sdh*B/C/D	Subunit B/C/D of the Succinate dehydrogenase gene
SDHI	Succinate dehydrogenase inhibitor
Sdh	Succinate dehydrogenase gene
UV-light	Ultraviolet light
WT	Wild type

Danksagung

Lieber Hans, vielen lieben Dank für die tolle Betreuung und das Vertrauen, dass du mir stets entgegengebracht hast. Danke für die großen Freiheiten in der Themengestaltung meiner Doktorarbeit und deine uneingeschränkte Unterstützung.

Lieber Ralph, vielen Dank, dass ich meine Dissertation an deinem Lehrstuhl machen durfte und du immer ein offenes Ohr für mich hattest.

Ganz besonders möchte ich mich bei Hans Sellmaier und Martin Köckeis, vom Gewächshauslaborzentrum in Dürnast, für die große Unterstützung bei meinen Feldversuchen bedanken. Ohne euch wären meine Versuche nicht durchführbar gewesen.

Ein großes Dankeschön gilt auch dem gesamten GHL für die tolle Unterstützung bei der Durchführung meiner Gewächshausversuche. Lieber Tom, dir möchte ich dabei besonders für deine tatkräftige Hilfe und deine liebevolle Pflege meiner Kartoffelpflanzen danken.

Bei Andrea Klaus und Regina Dittebrandt möchte ich mich für die viele Unterstützung im Labor bedanken.

Ich möchte mich auch bei all meinen Studenten bedanken, die über Forschungsprojekte, Bachelor- und Masterarbeiten oder als studentische Hilfskräfte wesentlich zum Erfolg dieser Arbeit beigetragen haben. Danke an Nicole Chaluppa, Nicole Klaus, Franziska Metz, Luisa Lehmann, Alexander Reinl, Stephanie Brüderle, Yvonne Katemann, Miriam Buksch und Carolina Honert.

Für die spannenden Projekte und die tolle Zusammenarbeit möchte ich mich besonders bei Frau Prof. Dr. Corinna Dawid und ihrem Doktoranden Sebastian Baur vom Lehrstuhl für Lebensmittelchemie und Molekulare Sensorik und bei Herrn Prof. Dr. Michael Rychlik und seiner Doktorandin Marina Gotthardt vom Lehrstuhl für Analytische Lebensmittelchemie bedanken.

Lieber Felix, ich möchte mich ganz herzlich für die schöne Zeit, den seelisch-moralischen Beistand und die vielen Gespräche bedanken, die auch mal über den Arbeitsalltag hinausgingen. Unsere fachlichen Diskussionen und deine Ratschläge habe ich immer sehr geschätzt.

Sehr herzlich möchte ich mich auch bei der gesamten Phyto-Familie für die freundliche Arbeitsatmosphäre bedanken. Ganz besonders möchte ich mich bei meinen Büro-Kollegen Dr. Tina Steidele, Dr. Alex Coleman und Julian Maroschek für den angenehmen Arbeitsalltag und eure Ratschläge bedanken.

Ein besonderer Dank gilt auch den guten Feen aus dem Sekretariat: Traudl Schröttle, Claudia Gün und Daniela Ingalls. Ohne euch wäre der Verwaltungsakt nicht zu bewältigen gewesen.

Vielen lieben Dank auch an meine Familie und Sebastian, dass ihr mich in allem uneingeschränkt unterstützt und immer für mich da seid.