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Rhizoctonia solani in sugar beet
–
Relations between soil physical properties and disease severity as well as quantification of the Rhizoctonia inoculum potential in soils

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Manuscript I

Einfluss der Bodenstruktur auf den Befall mit *Rhizoctonia solani* an Zuckerrüben (Beta vulgaris ssp. Vulgaris) – erste Ergebnisse

Manuscript II

Effect of Sugar Beet Variety and Nonhost Plant on *Rhizoctonia solani* AG2-2IIIB Soil Inoculum Potential Measured in Soil DNA Extracts

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List of abbreviations

Abb.	Abbildung
ANOVA	analysis of variance
ANCOVA	analysis of covariance
AG	anastomosis group or Anastomosegruppe
anf	anfällig
BS	Befallsstärke
BSA	Bundessortenamt
BZE	Bereinigter Zuckerertrag
Ct	cycle treshold
DNA	deoxyribonucleic acid
E	environment
ELISA	enzyme linked immunosorbent assay
EW	Eindringwiderstand
Fig.	figure
G	Genotyp
GM	grain maize
GPV	Gesamtporenvolumen
IP	inoculum potential
ITS	internal transcribed spacer
KM	Körnermais
LK	Luftkapazität
PCR	polymerase chain reaction
PDA	potato dextrose agar
PDB	potato dextrose broth
PL	Pneumatische Leitfähigkeit
QTLs	quantitative trait loci
res	resistant or resistent
rpm	rounds per minute
SB	sugar beet
SM	silage maize or Silomais
sus	susceptible
T	cultivation treatment
Tab.	table or Tabelle
V	variety
VB	Vorfrucht/Bodenbearb.
WSY	White sugar yield
ZR	Zuckerrübe

1. Prolog

***Rhizoctonia solani* in sugar beet: general information, economic importance and control measures**

1.1 Classification and distribution

The soil-borne and plant-pathogenic basidiomycete *Rhizoctonia solani* J. G. Kühn [teleomorph = *Tanatephorus cucumeris* Frank (Donk)] is a species complex and isolates are distributed world-wide in almost all arable soils (Sneh et al. 1996). The classification within this species complex is made by 13 anastomosis groups (AG) based on the ability of different isolates to fuse their hyphae (Anderson 1982; Carling 1996). Carling 1996 reported that a close genetic relation of two isolates can be determined by an almost perfect fusion of their hyphae, while less related isolates form imperfect fusions. Different AG infest different host plants whereby host ranges are broad and overlap across AG (Carling et al. 2002; Arakawa and Inagaki 2014). Some AG are further classified in subgroups (Ogoshi 1987; Carling 1996) depending on host specificity as well as genetic or biochemical characteristics (Cubeta and Vilgalys 1997).

For sugar beet, *R. solani* is the causal agent of the Rhizoctonia crown and root rot with AG2-2IIIB isolates identified to be the most aggressive subgroup in Germany (Führer Ithurrart 2003) and other Central European sugar beet production areas, e.g. Belgium (Coosemans et al. 2001), as well as in the USA (Bolton et al. 2010; Strausbaugh et al. 2011). Moreover, differences in pathogenicity between different isolates of AG2-2IIIB were observed in the past decades (Herr and Roberts 1980; O´Sullivan and Kavanagh 1991). Besides AG2-2IIIB, AG2-2IV was reported to cause crown and root rot symptoms in sugar beet in the United States (Engelkes and Windels 1996). Further, *R. solani* could also cause ‘seedling damping-off’ of sugar beet as shown by Hanson and McGrath (2011) and Matsui et al. (2013) for some isolates of AG2-2 and AG4 and by Bolton et al. (2010) for some AG1 isolates.

1.2 Infestation process and economic importance

The survival of *R. solani* in the soil is enabled by the formation of durable sclerotia or by mycelium in the soil (Jager et al. 1991; Sumner 1996). Under unfavorable conditions, formation of sclerotia (strong melanized hyphae) is most effective for a long-term survival

(Sumner 1996). In contrast, short-term survival occurs as mycelium bound to organic material in the soil (Keijer 1996; Dircks et al. 2014). Thus, there is a high risk of carryover by soil via agricultural machinery or by water, resulting in an increased distribution of *R. solani* within and between fields (MacNish et al. 1993) and also regions. For a fast distribution and host infestation, the temporal and spatial progress of mycelial growth of *R. solani* in the soil is of major importance. Hence, propagation processes are affected by soil conditions and the susceptibility of the crop cultivated (Bailey et al. 2000, Otten et al. 2001). For sugar beet cultivation, Naiki and Ui (1977) determined the majority of sclerotia of *R. solani* being formed on infested beets or on organic residues in the top 10 cm of the soil. Thereby, the amount of sclerotia was shown to be correlated with the severity of the disease (Naiki and Ui 1977).

Infection of a plant by *R. solani* generally is a combination of mechanical and biochemical processes (Keijer 1996; Gvozdeva et al. 2006): When *R. solani* reaches a host, the fungus forms a specific t-shaped mycelium and infection cushions for mechanical penetration into the host tissue (Ruppel 1973). Besides, enzymes, such as cellulase, cutinase, and pectinase, are built to degrade the cell wall of the host (Baker and Bateman 1978; Gvozdeva et al. 2006). *Rhizotonia solani* is a necrotrophic fungus and pathogenesis is accompanied by weakening of the host by toxins (Poland et al. 2009) and, finally, the death of the host tissue (Ruppel 1973; Weinhold and Sinclair 1996).

Due to the soil-borne nature of *R. solani*, disease symptoms on sugar beet fields mainly occur in patches (Herr 1996). Disease patches are highly mobile within fields and do not occur each year on the same place (Hyakumachi and Ui 1982). The first aboveground symptom, often not visible before canopy closure, is a stunted leaf growth with chlorosis of individual sugar beet plants. Later, a permanent wilting of the foliage is observed (Fig. 1A, B). Then, petioles become dark lesions and dead leaves form a black rosette around the beet crown (Fig. 1C). A general belowground symptom of Rhizoctonia crown and root rot is a dark brown to black

rotting of the root tissue. Typically, deep cracks become visible on the root or in the crown area. Further, the root can show sunken, dark brown to black colored lesions of different sizes (Fig. 1D). Thereby, the margin between infested and healthy root tissue is usually sharp and clear. Severe infestation leads to a complete shrinking and mummification of the root (Fig. 1E) (Halloin et al. 1999; Windels et al. 2009). Infested tissue is susceptible for further infections by other fungi such as *Fusarium oxysporum* and *Aphanomyces cochlioides*. (Harveson and Rush 1994, 1997) or bacteria such as Leuconostoc (Strausbaugh 2011) resulting in additional bacterial rot.

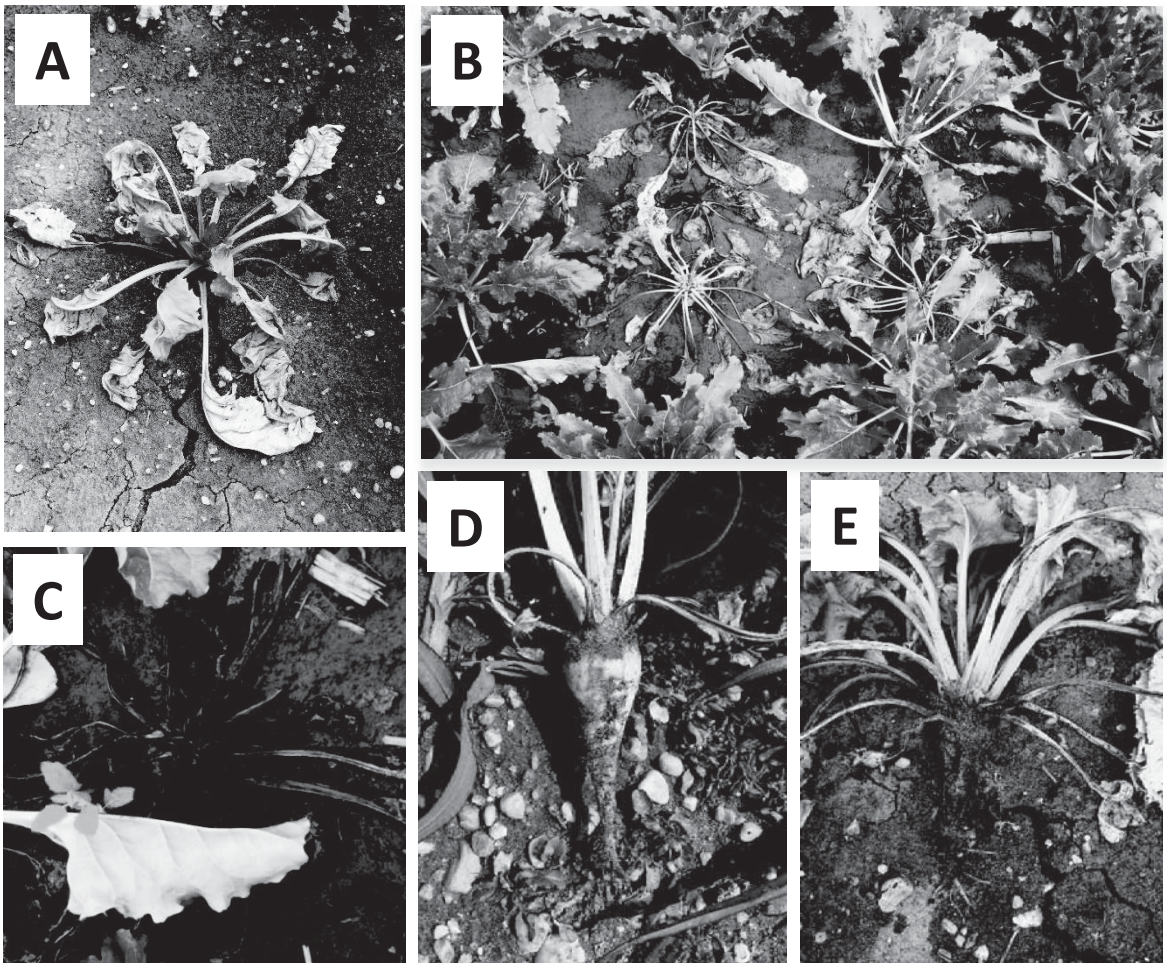

Fig. 1 Symptoms of Rhizoctonia crown and root rot on sugar beet in the field. A: First aboveground symptoms – stunted leaf growth and a dull leaf color, wilting. B: Typical disease patch with infested plants standing in close proximity to healthy plants (August). C: Black rosette of rotten leaves. D: Dark brown to black rotting of infested root tissue. E: Shrinking and mummification of the root. (Photos: Institute of Sugar Beet Research).

In German sugar beet production, severe *R. solani* infestation occurs on approximately 5% (10,000 ha) of the sugar beet fields (Büttner et al. 2002; Führer Ithurrart 2003), mainly within

four infestation areas (Fig. 2) in Lower Bavaria, the Rhineland, South Baden and near St. Michaelisdonn in Schleswig-Holstein (Büttner et al. 2002). A new estimate of the proportion of Rhizoctonia crown and root rot occurrence in Germany confirmed that in 2015, 10,000 ha, mainly located in Lower Bavaria and the Rhineland, were infested with the disease (personal communication, Dr. Erwin Ladewig, IfZ, July 2016). Throughout Europe, approximately 36,000 ha are infested (Garcia et al. 2001). In the USA, 50% of the sugar beet production areas are estimated to be of high risk for *R. solani* infection (Whitney and Duffus 1986) with yield losses of about 60% (Allen et al. 1985) and an overall economic damage of 2% per year (Kiewnick et al. 2001). Büttner et al. (2002) reported similar yield losses due to Rhizoctonia crown and root rot infection for Europe. Besides yield losses, the technical quality of infested sugar beet is decreased, leading to problems in further sugar beet processing, e.g. a higher amount of sugar in molasses that cannot be crystalized (Büttner et al. 2002; Bruhns et al. 2004).

1.3 Control of Rhizoctonia crown and root rot in sugar beet

1.3.1 Chemical and biological control measures

In Germany, fungicides for a chemical control are not registered with an indication for Rhizoctonia crown and root rot in sugar beet, whereas in the USA chemical control is one of the main measures to avoid a severe infection with *R. solani*.

Chemical control of can be performed by seed treatment (Windels and Brantner 1997, 2002, 2003) or by application of fungicides, within the 4-8 leaf stage, with Azoxystrobin (strobilurin) that has been reported to be the most effective active ingredient (Jacobsen et al. 1996, 1998; Kiewnick et al. 2001). Azoxystrobin is not mobile (no basipetal transfer) within the plant (Bartlett et al. 2001). Several studies demonstrated the efficacy of Azoxystrobin on *R. solani* when it was applied directly to the soil (Jacobsen et al. 2012; Khan and Hakk 2013). Furthermore, it was shown in experiments with artificial inoculation that yield losses can be reduced by more than 60% due to fungicide application (Kiewnick et al. 2001; Stump et al.

2002, 2004). In addition, first experiments to chemically control Rhizoctonia crown and root rot by a combination of Azoxystrobin and Difenoconazol in German sugar beet production areas demonstrated that disease severity of a susceptible sugar beet variety was decreased due to the fungicide compared to an untreated control and that the white sugar yield was increased significantly (Bartholomäus et al. 2016a). First reports of *R. solani* isolates with a reduced sensitivity against Strobilurins in rice and sugar beet (Olaya et al. 2013; Arabiat and Khan 2014) underline the importance of alternative active ingredients to avoid resistance formation due to chemical control of Rhizoctonia crown and root rot (Bolton et al. 2010).

Moreover, optimizing the time of application as well as the application method are important factors in an effective control of the disease by fungicides. Harveson (2009) and Jacobsen et al. (2004) showed that application simultaneously with inoculation of the plants resulted in lowest disease severity. Moreover, reduction of the disease severity and increase in the white sugar yield did not differ between a band and a broadcast application (Brantner and Windels 2010; Khan and Carlson 2011)

Biological control of Rhizoctonia crown and root rot could be another measure in terms of an integrated control strategy. Despite the positive effect of antagonistic of fungal and bacterial antagonists like *Trichoderma* spp. (Thornton and Gilligan 1999; Asram-Amal et al. 2005; Grosch et al. 2006), *Verticillium* spp. (Velvis et al. 1989) or *Pseudomonas* spp. (Thrane et al. 2001; Kai et al. 2007) was shown, the effectiveness of antagonistic microorganisms was never demonstrated under field conditions.

Trichoderma spp. and *Verticillium* spp. are micro-parasites that, directly infest *R. solani* hyphae (Chet and Baker 1980, Velvis et al. 1989; Grosch et al. 2006) or secret fungistatic substances (Grosch et al. 2006). Bacterial antagonists like *Pseudomonas* species secret antibiotic substances resulting in an inhibited spread of *R. solani* in the soil (Thomashow et al. 1990; Thrane et al. 2001; Nielsen et al. 2002; Grosch et al. 2005; Kai et al. 2007).

Furthermore, research was done with binucleate and non-pathogenic isolates of *R. solani* (Herr, 1988; Sumner and Bell 1994; Jabaji-Hare et al. 1999; Khan et al. 2005) or hypovirulate isolates (Bandy and Tavantzis 1990; Sneh et al. 2004) to control *R. solani*-caused diseases of different crops due to a competition for resources.

1.3.2 Agronomic control measures

The choice of the sugar beet variety is an important agronomic measure directly affecting disease severity and occurrence of Rhizoctonia crown and root rot: It was shown that susceptibility against *R. solani* varies across sugar beet varieties (Buddemeyer et al. 2004; Buddemeyer and Märländer 2005; Buhre et al. 2009) and that cultivation of less susceptible varieties is the most suitable measure against Rhizoctonia crown and root rot (Buddemeyer and Märländer 2005). It was demonstrated that resistant sugar beet varieties show a reduced disease severity compared to susceptible varieties, however, the yield performance of resistant varieties is declined under non-diseased conditions (Buddemeyer and Märländer 2005). Therefore, those varieties are only cultivated to a low extend on fields or areas with a high disease pressure. Due to the fact that resistance against *R. solani* is oligogenetic, breeding of resistant sugar beet varieties is challenging (Hecker and Ruppel 1975; Lein et al. 2008) and, therefore, mechanisms of resistance remain unclear (Büttner et al. 2002). Nevertheless, different quantitative trait loci (QTLs) were identified for a marker-assisted selection to improve resistance breeding, however, they only explained 71% of the phenotypic variance leading to the assumption that other minor QTLs are involved in resistance against *R. solani* (Lein et al. 2008).

Besides the sugar beet variety, the crop rotation is one of the most important agronomic factor affecting disease severity of sugar beet. Therefore, intensive research was done to evaluate the effect of different preceding crops on disease severity of Rhizoctonia crown and root rot on subsequently cultivated sugar beet. It was shown that an increased frequency of host crops,

such as maize, sorghum, alfalfa, soybean, or other bean species, within sugar beet crop rotations increases the disease severity of sugar beet (Rush and Winter 1994; Engelkes and Windels 1996; Buddemeyer and Märländer 2004; Garbeva et al. 2006; Buhre et al. 2009; Kluth and Varrelmann 2010). Thus, avoiding the cultivation of host plants within sugar beet crop rotations is recommended (Engelkes and Windels 1996). A survey of farmers from Lower Bavaria (Germany) revealed that an infection of sugar beet with Rhizoctonia crown and root rot was more abundant on farms with a high proportion of sugar beet and maize within the crop rotation (Bürcky and Zellner 2000). Thus, in Germany maize is the host plant with the strongest agronomic relevance. Führer Ithurrart (2003) was one of the first indicating its significant contribution to *R. solani* infection of sugar beet, but susceptibility to *R. solani* was variable between maize varieties (Pfähler and Petersen 2004; Windels et al. 2008; Kluth and Varrelmann 2010).

Overall, crop rotation design can contribute to improve the control of the disease and was shown to be one of the most effective measures. Buhre et al. (2009) and Kluth et al. (2010) demonstrated a positive effect of intercrops as well as crop rotations with a high proportion of nonhost crops on disease severity of subsequent sugar beet. Larkin and Honeycutt (2006) showed that cultivation of nonhost plants, e.g. barley and canola, within a 3-year-cropping system decreased severity of *R. solani* –caused diseases (stem and stolon canker and black scurf) in subsequently grown potato due to a higher microbial activity in the soil. A further factor that was shown to increase the Rhizoctonia crown and root rot disease severity in sugar beet is the amount of host plant residues, e.g. sugar beet leaves and maize residues, remaining in the field (Dircks et al. 2014). An increase of the disease severity in sugar beet was not observed when residues of winter wheat remained in the field. In addition, Ruppel (1985) reported that residues of sorghum and beans also increased disease severity in sugar beet in contrast to barley residues that were not conducive to *R. solani*.

Soil physical properties are known as driver of *R. solani*-caused diseases of several host crops, especially in interaction with climatic conditions. Besides site-inherent conditions, soil physical properties are mainly affected by soil tillage practices and, therefore, studies often differentiate soil tillage: Rovira (1986), Pumphrey et al. (1987), and Paulitz (2006) revealed an increase in the severity of disease caused by *R. solani* in wheat (e.g. for bare patch and root rot) due to conservation tillage practices. In contrast, the retrieval of *R. solani* from root tissue of spring barley and soybean was higher from direct drilled compared to conservation tilled soil, probably due to a promotion of antagonistic microorganisms in the soil by plowing (Sturz and Carter 1995). Further, Schroeder and Paulitz (2008) found no differences in disease incidence and severity of cereal crops between tilled and direct-seeded fields. For sugar beet it was shown that conventional tillage by plowing only reduced disease severity under strong disease-promoting conditions, e.g. after two-times cultivation of maize (Buhre et al. 2009).

In contrast to conventional tillage, conservation tillage is characterized by a lower working depth (10-15 cm compared to 20-30 cm for conventional tillage), resulting in a lower intensity of soil loosening and mixing and a higher concentration of organic debris in the topsoil (Frede et al. 1994), higher amounts of nutrients and an increased microbial activity (Sumner et al. 1981, Sturz et al. 1997). Further, conservation tillage results in a higher bulk density, being indicative for compacted soils, with a reduced air-filled pore volume and reduced air and water conductivity resulting in an impeded soil warming (Johnson and Lowery 1985). In general, soil compaction reduces sugar beet shoot and root growth (Hoffmann and Jungk 1995) and reduces plant population as well as root length and distribution (Brereton et al. 1986). Thus, soil physical properties as provoked by conservation tillage, may lead to substantial yield losses of sugar beet (Wolf and Verreet 1999; Tomanova et al. 2006; Koch et al. 2009). Studies by Kühn et al. (2009) demonstrated that soil texture, carbonate content, pH value, redox potential as well as P and K content of the soil do not affect the occurrence and

severity of Rhizoctonia crown and root rot in sugar beet. However, they found that the C/N ratio was higher in diseased compared with healthy parts of the field.

The promotion of diseases severity due to compacted soils, often occurring in the headlands (Hanus and Horn 1992), was described previously for white bean (Tu and Tan 1991) and sugar beet (Buddemeyer and Märländer 2004). On the other hand, non-compacted soils, with continuous air-filled pores, enhance the spatial distribution of *R. solani* (Glenn & Sivasithamparam 1990; Otten & Gilligan 2006). Moreover, conservation tillage can decrease soil temperature (Johnson and Lowery 1985) and can also possibly reduce infestation since it was shown that disease severity increased with increasing temperature. *Rhizoctonia solani* is favored by temperatures above 20 °C (Baker and Martinson 1970; Engelkes and Windels 1994; Wolf and Verreet 1999; Zens et al. 2002; Kirk et al. 2008). Rhizoctonia crown and root rot symptoms started to occur even at low soil temperatures of 15 °C (Bolton et al. 2010). Besides soil temperature, a high soil moisture or waterlogging was reported to increase Rhizoctonia crown and root rot disease severity of sugar beet (Wolf and Verreet 1999; Kiewnick et al. 2001; Führer Ithurrart et al. 2004).

Overall, it is not clear to which extend soil tillage affect the expression of the disease as well as resulting yield losses and therefore its use as control measure remained unclear. Furthermore, the importance of soil structure and, more specifically, individual soil physical parameters for Rhizoctonia crown and root rot disease severity of sugar beet remained unexplained till now.

1.4 Detection and quantification of *R. solani* in soils

In general, detection and quantification of soil-borne pathogens is challenging due to their heterogeneous spatial distribution within soils. Different methods to detect and quantify *R. solani* were developed during the past decades. The close association of *R. solani* with soil organic material, e.g. plant residues, led to methods using elutriation (Clark et al. 1978), wet-

sieving (Weinhold 1977; Clark et al. 1978), or dry-sieving (Papavizas 1968). Those methods aim to remove the organic particles containing the pathogen which are then suspended in water agar. Subsequently, identification was performed by plating the colonies on potato dextrose agar. Other methodological approaches were direct microscopy of pathogen propagules associated with plant debris (Boosalis and Scharen 1959) or baiting methods. For baiting, plant residues or susceptible host plants (Papavizas and Davey 1959, 1962) as well as toothpicks (Paulitz and Schroeder 2005) or nylon nets (Neate and Benger 1995) were used to isolate *R. solani* from soil samples for further identification. Different baits showed different detection accuracy and baiting by host plants mainly detects the favored isolates of the current host, whereas non-specific baits mainly detect saprophytic isolates (van Bruggen and Arneson 1986). Methods for further identification of pathogens are the use of ELISA tests (Otten et al. 1997; Thornton et al. 1999), the detection by specific DNA probes (Whisson et al. 1995), or PCR-based procedures with pathogen-specific primers (Salazar et al. 2000; Budge et al. 2009; Woodhall et al. 2012, 2013).

Real-time PCR based quantification techniques allow a rapid and definite identification and ensure a high accuracy compared to sieving and baiting techniques (Woodhall et al. 2012). A recent approach by Boine et al. (2014) combined baiting of *R. solani* from soil samples by sterilized quinoa grains and following real-time PCR based quantification of *R. solani* DNA extracted from grains infested. Actually, different *R. solani* AG, e.g. AG3 (Lees et al. 2002) or AG8 and AG10 (Okubara et al. 2008), were successfully detected and quantified by real-time PCR based approaches after DNA extraction from soil. However, there was no reliable technique for a direct extraction of DNA from soils and quantification of the soil inoculum potential of *R. solani* AG2-2IIIB so far.

1.5 Research objectives and structure of the thesis

The effect of different soil tillage practices on the occurrence of the Rhizoctonia root and crown rot is controversially discussed in literature. Moreover, there is no soil physical

parameter identified so far which closely correlates with the disease severity in sugar beet. Due to this lack of knowledge, this study aimed to identify individual soil physical properties with a significant impact on disease severity of Rhizoctonia crown and root rot in sugar beet grown in the field. Besides, the effect of agronomic measures, such as soil tillage practices, sugar beet variety type and amount of organic residues, on disease severity was also of special interest. Soil structural properties were differentiated (i) by growing maize as a *R. solani* - susceptible preceding crop before sugar beet to vary the amount of maize residues (silage maize, grain maize) and (ii) by varying soil tillage (plowing, mixing tillage with a cultivator, shallow cultivation after soil compaction by wheeling) in two-factorial field trials in two German sugar beet production areas (Lower Saxony and Lower Bavaria) in 2013-14 and 2014-15. Variation of disease severity and yield was caused by cultivation of a susceptible and a resistant sugar beet variety. Furthermore, we saw a need to determine the *R. solani* inoculum potential directly from the soil and therefore a new reliable real-time PCR based quantification method was developed. We applied the method by studying different sugar beet variety types and a winter rye as a nonhost plant on the soil inoculum potential.

This cumulative doctoral thesis consists of the following three manuscripts that are already published or submitted for publication in scientific journals:

Manuscript I, „Einfluss der Bodenstruktur auf den Befall mit *Rhizoctonia solani* an Zuckerrüben (*Beta vulgaris* ssp. *Vulgaris*) – erste Ergebnisse“ (Schulze et al. 2016a), published in the journal *Sugar Industry*, demonstrates first results of the impact of penetration resistance, pore volume, air capacity and pneumatic conductivity on white sugar yield and disease severity of a susceptible and a resistant sugar beet variety. Further, the effect of different soil tillage treatments on white sugar yield and disease severity is discussed.

In manuscript II, ‘Effect of Sugar Beet Variety and Nonhost Plant on *Rhizoctonia solani* AG2-2IIIB Soil Inoculum Potential Measured in Soil DNA Extracts’ (Schulze et al. 2016b), published in the journal *Phytopathology*, we described the methodological approach to

quantify *R. solani* in soil samples by real-time PCR. Further, we demonstrated for the first time the effect of the sugar beet variety type and the nonhost crop winter rye on the *R. solani* soil inoculum potential.

In manuscript III, 'Relationships between varying soil tillage practices causing different soil physical properties and disease severity of *Rhizoctonia solani* in two sugar beet (*Beta vulgaris* L.) varieties' (Schulze et al. 2016c), submitted to *Plant and Soil*, we demonstrated that the penetration resistance is the major soil physical parameter affecting white sugar yield and disease severity of sugar beet. Thus, maintaining a good soil structure is an important measure in disease control.

The epilog generally comprises: 'Integrated control of Rhizoctonia crown and root rot in sugar beet' with updated information for agricultural practice derived from this thesis project.

2. Manuscript I

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Einfluss der Bodenstruktur auf den Befall mit *Rhizoctonia solani* an Zuckerrüben (Beta vulgaris ssp. Vulgaris) – erste Ergebnisse

Impact of soil structural characteristics on *Rhizoctonia solani* infestation of sugar beet (*Beta vulgaris* ssp. *vulgaris*) - first results

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Zusammenfassung

Der bodenbürtige Pilz *Rhizoctonia solani* Kühn ist der Erreger der Späten Rübenfäule der Zuckerrübe und kann erhebliche Ertragsverluste verursachen. Krankheitsauftreten und Befallsstärke sind neben dem Zuckerrübengenotyp und der Vorfrucht abhängig von spezifischen bodenstrukturellen Eigenschaften. In den vorgestellten Versuchen wurde die Bodenstruktur durch eine variierte Bodenbearbeitung (Pflug, Grubber, Überrollen und flache Grubberbearbeitung) differenziert. Zwei Zuckerrübengenotypen (anfällig, resistent) wurden nach Mais angebaut. Aufgrund eines insgesamt niedrigen Befallsniveaus mit *R. solani* an Zuckerrüben konnten keine Zusammenhänge zwischen der Befallsstärke von *R. solani* und den untersuchten Bodenparametern (Eindringwiderstand, Gesamtporenvolumen, Luftkapazität, Pneumatische Leitfähigkeit) festgestellt werden. Darüber hinaus gab es keine Befallsunterschiede zwischen den Bodenbearbeitungsvarianten. Die Ergebnisse lassen allerdings vermuten, dass reduzierte Bodenbearbeitung zu einem höheren Befall mit *R. solani* als konventionelle Pflugbearbeitung führen könnte. Kam es zum Befall, war dieser beim anfälligen Genotyp stärker ausgeprägt als beim resistenten Genotyp.

Schlagwörter: Bodenbearbeitung, Bodenstruktur, *Rhizoctonia solani*, Zuckerrübe

Abstract

The soil-borne fungus *Rhizoctonia solani* Kühn is known as causal agent of the Late Root and Crown Rot of sugar beet and can lead to enormous yield losses. Disease occurrence and severity are affected by the sugar beet genotype and the pre-crop but also by soil structural characteristics. In this study the soil structure at the trial was differentiated by a variation of soil tillage (plow, cultivator, wheeling and shallow cultivation). Two sugar beet genotypes (susceptible, resistant) were grown after maize. Due to a low disease severity no correlations were found between the disease severity and the investigated soil characteristics (penetration resistance, air capacity, pore volume, pneumatic conductivity). Furthermore, the tillage

treatments caused no differences in disease incidence and severity. However, the results allow the presumption that reduced tillage could increase *R. solani* infestation compared to conventional plowing. The susceptible genotype showed a higher disease severity compared to the resistant genotype if infestation occurred.

Keywords: Soil tillage, soil structure, *Rhizoctonia solani*, sugar beet

1. Einleitung

Die Späte Rübenfäule der Zuckerrübe (*Beta vulgaris* ssp. *vulgaris*) tritt in Deutschland zunehmend auf, wobei die Regionen Niederbayern und Rheinland vorwiegend betroffen sind. Verursacht wird die Krankheit durch das bodenbürtige Pathogen *Rhizoctonia solani* Kühn. *Rhizoctonia solani* ist ein Pilzkomplex, dessen genetisch unterschiedliche Spezies zur Klassifizierung in Anastomosegruppen (AG) unterteilt werden (*Ogoshi*, 1987), wobei innerhalb einer AG eine weitere Unterscheidung zwischen verschiedenen Untergruppen möglich ist. Für die Späte Rübenfäule ist in Deutschland maßgeblich die AG2-2IIIB verantwortlich (*Büttner* et al., 2002). In den USA wurde auch AG2-2IV als Erreger der Späten Rübenfäule identifiziert (*Allen* et al. 1985). Neben der Zuckerrübe sind weitere Kulturpflanzen als Wirt bekannt, darunter Soja, Reis und Mais (*Engelkes* und *Windels*, 1996). *Rhizoctonia solani* ist persistent, einerseits durch saprophytisches Pilzmyzel, welches mit der organischen Substanz im Boden assoziiert ist, und andererseits durch die Bildung von Sklerotien zur mehrjährigen Überdauerung. Charakteristisch für die Späte Rübenfäule ist, dass sie im Feld nesterweise auftritt, wobei sowohl die Befallshäufigkeit als auch die Befallsstärke innerhalb eines Feldes sowie zwischen Feldern sehr stark variieren kann (*Herr*, 1996).

Wie von *Anees* et al. (2009) zusammengefasst, ist das Inokulumpotenzial im Boden und somit die potenzielle Gefahr des Auftretens der Krankheit sowie die Befallsstärke von

Umweltfaktoren wie Temperatur und Bodenfeuchte, der Häufigkeit des Anbaus von Wirtspflanzen und den Resistenzeigenschaften der angebauten Zuckerrübensorte abhängig. Der Anbau von gegenüber *R. solani* resistenten Zuckerrübensorten ist in Befallsregionen weit verbreitet und häufig die einzige Möglichkeit, einem Befall mit *R. solani* und einem daraus resultierenden Ertragsverlust entgegenzuwirken. Resistente Sorten ermöglichen eine verminderte Symptomausprägung und eine stabilere Ertragsleistung unter Befall, zeigen bei Nichtbefall jedoch Mindererträge im Vergleich zu anfälligen Sorten (*Büttner* et al., 2002; *Buddemeyer* und *Märländer*, 2005). Fungizide zur Bekämpfung der Späten Rübenfäule sind in Deutschland gegenwärtig nicht zugelassen. Deshalb wird ackerbaulichen Kontrollmaßnahmen, wie z. B. der Häufigkeit des Anbaus von Wirtspflanzen in der Fruchtfolge, der Gestaltung der Bodenbearbeitung und dem Ernterestmanagement große Bedeutung zugeschrieben, wenngleich die Mechanismen ihrer Wirkung sowie deren Höhe und Zuverlässigkeit nicht sicher einzuschätzen sind. Ein hoher Anteil *R. solani*-anfälliger Wirtspflanzen in der Fruchtfolge, vor allem Mais, führte bei nachfolgend angebauten Zuckerrüben zu erhöhtem Befall und vermindertem Ertrag (*Führer Ithurrart* et al., 2004; *Buhre* et al., 2009; *Kluth* und *Varrelmann*, 2010). Auch der Anbau anfälliger Zwischenfrüchte resultierte in höherem Befall bei der nachfolgenden Zuckerrübe (*Kluth* et al., 2010). Zur Wirkung von im Feld verbleibenden Ernteresten von Wirtspflanzen wurde bislang beobachtet, dass sich diese ebenfalls negativ auf nachfolgende Zuckerrüben auswirkten und den Befall mit *R. solani* erhöhten (*Ruppel*, 1985; *Dircks* et al., 2014).

Von den Umweltfaktoren haben Bodentemperatur und -feuchte vermutlich großen Einfluss auf die Befallsstärke der Späten Rübenfäule, wenngleich deren Einfluss bislang nur in Gewächshausversuchen eindeutig nachgewiesen wurde (*Bolton* et al., 2010). Im Feld wurde der direkte Einfluss von Kenngrößen der Bodenstruktur sowohl auf die Befallshäufigkeit als auch auf die Befallsstärke bisher wenig untersucht. Zum Einfluss der Bodenbearbeitung auf einen Befall von *R. solani* sind demgegenüber zahlreiche Arbeiten bekannt. So fanden *Buhre*

et al. (2009) heraus, dass sich in Mais-Mais-Zuckerrübe-Fruchtfolgen eine Pflugbearbeitung vor der Zuckerrübenaussaat im Vergleich zur Mulchsaat mindernd auf den Befall mit *R. solani* auswirkte. Darüber hinaus zeigte sich nach dem Überrollen mit schweren Maschinen ein höherer Befall auf verdichteten Böden mit höheren Lagerungsdichten im Vergleich zu nicht überrollten Böden (*Buddemeyer* und *Märländer*, 2004). In einer anderen Arbeit fanden *Kühn* et al. (2009) keine Beziehung zwischen den abiotischen Bodenkenngrößen Nährstoffgehalt, pH-Wert, Bodentextur und Trockenrohdichte und dem Auftreten der Späten Rübenfäule auf Praxisschlägen. Es wurde lediglich ein signifikant niedrigeres C/N Verhältnis in Befallsnestern verglichen mit befallsfreien Teilflächen festgestellt.

Ziel dieser Arbeit war es, den Einfluss unterschiedlicher Ausprägungen der Bodenstruktur auf die Befallsstärke von *R. solani* in Zuckerrüben zu untersuchen. Dazu wurden an zwei Standorten mit (Haardorf [Niederbayern]) und ohne (Göttingen [Niedersachsen]) vorherigem Befall mit *R. solani* Feldversuche durchgeführt, in denen die Bodenstruktur durch unterschiedliche Maßnahmen der Bodenbearbeitung variiert wurde. Kenngrößen der Bodenstruktur wurden an ungestörten Bodenproben gemessen. Die Versuchsflächen wurden künstlich inokuliert und das Inokulumpotenzial sollte durch den Anbau der Wirtspflanze Mais vor der Prüffrucht Zuckerrübe homogenisiert werden. Es wurden zwei unterschiedliche Zuckerrübengenotypen (anfällig, resistent) angebaut, an denen die Befallsstärke (BS) von *R. solani* durch Bonituren erfasst wurde.

2 Material und Methoden

2.1 Versuchsanalage und -durchführung

Die Feldversuche wurden im Versuchsjahr 2013/14 in Göttingen (Gö14; 51°33‘04.41“ N 9°54‘0.49“ O) und Haardorf (Ha14; 48°43‘07.47“ N 12°59‘07.98“ O) angelegt. Die Umwelt Ha14 repräsentiert hierbei einen natürlichen Befallsstandort von *R. solani* in Niederbayern. In Gö14 wurde ein natürlicher Befall mit *R. solani* in der Vergangenheit nicht beobachtet. Die

Bodenart ist in Gö14 als Ut3 (14.1 % Ton, 75.5 % Schluff) und in Ha14 als Ut4 (19.2 % Ton, 71.5 % Schluff) klassifiziert worden (Ad-hoc-AG Boden, 2005). Zur Variation der im Feld verbleibenden Ernterestmengen wurde als Vorfrucht an beiden Standorten auf Teilflächen Silomais (SM) bzw. Körnermais (KM) angebaut. Beide Versuchsflächen wurden vor der Maisaussaat ganzflächig mit einem Gersteninokulum inokuliert, um das Inokulumpotenzial im Boden zu erhöhen (Gö14), bzw. zu homogenisieren (Ha14). Nach der Maisernte wurde die Bodenbearbeitung variiert, um unterschiedliche Bodenstrukturzustände zu erzeugen. Bodenbearbeitungsmaßnahmen waren eine wendende Pflugbearbeitung mit 25 cm Bearbeitungstiefe (P25), eine nicht-wendende Grubberbearbeitung mit 10 cm Bearbeitungstiefe (C10) sowie eine 5 cm flache Grubberbearbeitung (C5), der eine Überrollung (Spur an Spur) mit schweren landwirtschaftlichen Maschinen (Masse 8,6 t, Reifeninnendruck vorne 2 bar, hinten 2,5 bar, Radlast, 2,2 t) vorgeschaltet war. Im darauffolgenden Frühjahr wurden eine anfällige (ZR 1988, Ausprägungsstufen für den Ertrag nach Bundessortenamt, 2014: Bereinigter Zuckerertrag BZE1 = 6, BZE2 = 6) und eine resistente (ZR 1555, Ausprägungsstufen für den Ertrag nach Bundessortenamt, 2014: BZE1 = 3, BZE2 = 3) Zuckerrübensorte angebaut (Kennnummern Bundessortenamt, 2014).

Die Abstufungen der Faktoren Bodenbearbeitung und Vorfrucht wurden zu den Haupteinheiten der eingerichteten Spaltanlage kombiniert. Da bei der nur flachen Bodenbearbeitungsvariante C5 die große Strohmenge der Vorfrucht KM erhebliche Probleme bei der Zuckerrübenaussaat erwarten ließ, wurde hier nur die Vorfruchtvariante SM durchgeführt. Untereinheit war die Zuckerrübensorte.

Es wurden vier Wiederholungen angelegt. In beiden Umwelten resultierte der Versuchsaufbau in 40 Parzellen mit jeweils 12 Reihen Zuckerrüben pro Parzelle und einer Länge von 14 m sowie einem Reihenabstand von 0,45 m. Die Zuckerrüben wurden nach dem Auflaufen von 7 cm auf 21 cm Abstand innerhalb der Reihen vereinzelt, um einen homogenen Pflanzenbestand sicherzustellen. Die Böden in Gö14 und Ha14 wiesen einen Gehalt an

Grundnährstoffen in der Gehaltsklasse C oder höher auf (Landwirtschaftskammer Niedersachsen, 2014). Die N-Düngung betrug einheitlich 110 (Gö14) bzw. 120 kg N ha^{-1} (Ha14) in allen Varianten. Pflanzenschutzmaßnahmen gegen Unkräuter, Schädlinge und Blattkrankheiten wurden nach regionalen Standards durchgeführt.

2.2 Zuckerrübenernte und Ertragsbestimmung

Im Oktober 2014 wurden pro Parzelle die mittleren zwei Zuckerrübenreihen (jeweils 28 laufende Meter) getrennt nach Blatt und Rübe geerntet. Die geernteten Zuckerrüben wurden in der Rübenwäsche des Instituts für Zuckerrübenforschung (IfZ) gewaschen, gewogen und zu Brei verarbeitet. Der Brei wurde schockgefroren und bis zur Analyse bei -18 °C gelagert. Die Bestimmung des Zuckergehalts des Rübenbreis erfolgte polarimetrisch. Die Melassebildner Kalium, Natrium und Amino-N wurden im aluminiumgeklärten Extrakt flammenphotometrisch (Kalium, Natrium) bzw. absorptionsphotometrisch (Amino-N) gemessen und zur Berechnung des Bereinigten Zuckergehaltes verwendet (*Hoffmann*, 2006). Der BZE wurde aus dem Rübenertrag und dem Bereinigten Zuckergehalt berechnet (*Märländer* et al., 2003).

2.3 Bonitur der Befallsstärke von *Rhizoctonia solani*

Die BS der Zuckerrüben mit *R. solani* wurde nach dem Waschen wie im Folgenden beschrieben bonitiert und als prozentualer Anteil befallener Zuckerrübenoberfläche ausgedrückt: im Bereich niedriger BS wurde zwischen einer BS von 0 % (gesunde Zuckerrübe, kein Befall), 2 % (erste kleine schwarze Läsionen am Rübenkörper) und 5 % (erste deutlich sichtbare Läsionen von mindestens 0,5 cm Durchmesser) unterschieden, um eine Differenzierung zwischen gesunden Zuckerrüben und erster Symptomausprägung sicherzustellen. Bei BS > 10 % erfolgte die Bonitur in 10-%-Schritten bis zu einem maximalen Befall mit *R. solani* von 100 % (komplett verrottete, mumifizierte Zuckerrübe).

Aus den Boniturwerten der einzelnen Zuckerrüben wurde ein mittlerer Wert für die BS jeder Parzelle berechnet.

2.4 Bodenparameter

Der Eindringwiderstand (EW) des Bodens bis 40 cm Tiefe wurde kurz nach der Aussaat (April) mit einem Penetrologger (Eijkelkamp, Giesbeek, NL; Messspitze 1 cm2 Fläche bei 60° Anstellwinkel) gemessen. In jeder Parzelle der Kombination Vorfrucht und Bodenbearbeitung wurde 10 Messwiederholungen in einer diagonalen Linie durchgeführt. Zur Bestimmung weiterer Kenngrößen der Bodenstruktur wurden ungestörte Bodenproben (Stechzylinder: 250 cm3, 80 mm Durchmesser, 10 Wiederholungen pro Parzelle Vorfrucht und Bodenbearbeitung) vertikal aus einer Bodentiefe von 7–12 cm entnommen. Die Stechzylinder wurden im Sandbett aufgesättigt und auf eine Saugspannung von 6,2 kPa (pF 1,8, Feldkapazität) eingestellt. Anschließend wurde die pneumatische Leitfähigkeit (PL) mit einem PL-Messgerät (UGT, Müncheberg) bestimmt. Nachfolgend wurden die Proben zur Erfassung des Wassergehaltes bei Feldkapazität gewogen, bei 105 °C bis zur Massenkonstanz getrocknet und abschließend zur Trockenmassebestimmung erneut gewogen. Die Bestimmung des Gesamtporenvolumens (GPV) erfolgte aus der zuvor berechneten Trockenrohdichte (Trockenmasse dividiert durch das Stechzylindervolumen) und der Dichte der Festsubstanz des Bodens, für die ein Wert von 2,65 g cm3 (Quarz) angenommen wurde. Die Luftkapazität (LK) wurde als Differenz aus GPV und Feldkapazität errechnet.

2.5 Statistische Auswertung

Die statistische Auswertung erfolge mit der Software R (R Core Team, 2014). Die Daten wurden mit der Funktion ‚shapiro. test‘ (Shapiro-Wilk-Test) auf Normalverteilung getestet. Die varianzanalytische Auswertung erfolgte mittels gemischter Modelle, die die Faktoren Vorfrucht/Bodenbearbeitung und Genotyp und deren Wechselwirkung als feste Effekte sowie Vorfrucht/Bodenbearbeitung geschachtelt in Wiederholung als zufällige Effekte beinhalteten.

Zur Berechnung wurde die Funktion ‚lme' aus dem Paket ‚nlme' (*Pinheiro* et al., 2013) verwendet:

Modell A

$$Bereinigter\ Zuckerertrag = VB + G + (VB\ x\ G)$$

$$Befallsstärke = VB + G + (VB\ x\ G)$$

VB = Vorfrucht/Bodenbearbeitung, G = Genotyp, VB x G = Interaktion zwischen VB und G

Da die Interaktion zwischen Vorfrucht/Bodenbearbeitung und Genotyp nicht signifikant war, wurde diese aus dem Modell entfernt:

Modell B

$$Bereinigter\ Zuckerertrag = VB + G$$

$$Befallsstärke = VB + G$$

VB = Vorfrucht/Bodenbearbeitung, G = Genotyp, VB x G = Interaktion zwischen VB und G

Anschließend wurde mittels der in der ‚lme' Funktion implementierten ‚ML'-Methode (maximum likelihood) und einer ANOVA getestet, ob das Entfernen des nicht signifikanten Interaktionsterms aus Modell A zu einer signifikanten Verbesserung des Modells führte. Danach wurden mit der ‚sp.plot'- und der ‚LSD.test'-Funktion ($p < 0{,}05$) aus dem Paket ‚agricolae' (*de Mendiburu*, 2013) signifikante Unterschiede ermittelt. Rangkorrelationskoeffizienten nach *Spearman* wurden mit Hilfe der Funktion ‚rcorr' aus dem Paket ‚Hmisc' (*Harrell*, 2015) berechnet und lineare Regressionen mit der Funktion ‚lm' angepasst. Die grafische Darstellung der Ergebnisse erfolge ebenfalls mit der Software R.

3 Ergebnisse

3.1 Bodenstruktur

Der EW an der Bodenoberfläche lag in allen Bodenbearbeitungsvarianten in Gö14 bei etwa 0,8–1,0 MPa (Abb. 1A). Ab 10 cm Tiefe kam es zu einer Differenzierung des EW zwischen den Bodenbearbeitungsvarianten in der Reihenfolge P25 < C10 < C5, wobei die

Standardabweichungen deutliche Überschneidungen im EW zwischen C10 und C5 aufzeigen. Die Bodenbearbeitungsvariante C5 erreichte in etwa 10 cm Tiefe einen EW von 1,8 MPa. Der weitere Verlauf des EW war in allen drei Bodenbearbeitungsvarianten bis 20 cm Tiefe konstant und stieg anschließend in P25 und C10 an. Ab 30 cm Tiefe (Pflugsohle) lag der EW in allen drei Bodenbearbeitungsvarianten bei > 1,5 MPa.

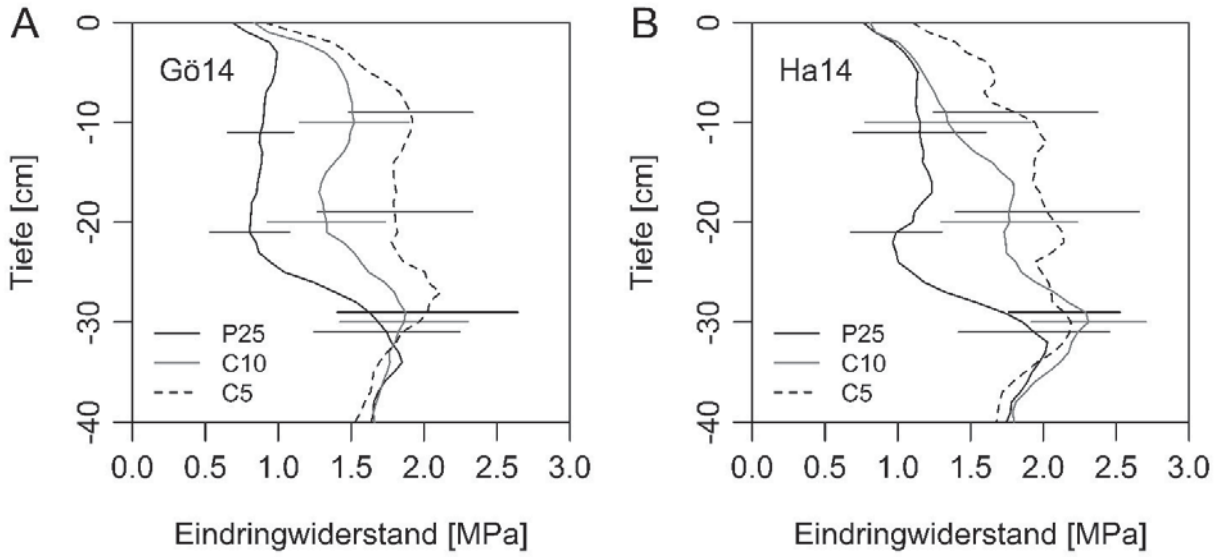

Abb. 1 Einfluss differenzierter Bodenbearbeitung auf den Eindringwiderstand des Bodens in MPa in 0-40 cm Tiefe nach der Zuckerrübenaussaat im April 2014 in den Umwelten Göttingen (Gö14) (A) und Haardorf (Ha14) (B). P25: Pflug 25 cm tief (n = 80). C10: Grubber 10 cm tief (n = 80). C5: Grubber 5 cm tief nach vorheriger Überrollung (n = 40). Balken zeigen die Standardabweichung.

In Ha14 war der EW an der Bodenoberfläche bei C5 höher als bei P25 und C10 (Abb. 1B). In 10 cm Tiefe gab es zwischen P25 und C10 lediglich einen geringen Unterschied, während der EW der Bodenbearbeitungsvariante C5 hier über 1,8 MPa lag. Zu berücksichtigen bleibt, dass die hohe Standardabweichung auch in Ha14 deutliche Überschneidungen des EW zwischen den drei Varianten aufzeigte. In etwa 20 cm Bodentiefe gab es eine deutlichere Differenzierung zwischen den Bodenbearbeitungsvarianten in der Reihenfolge P25 < C10 < C5. Im Bereich von etwa 20–30 cm Tiefe stieg der EW bei P25 und bei C10 weiter an. Der EW in 30 cm Tiefe lag für alle drei Bodenbearbeitungsvarianten über 1,8 MPa.

Im Mittel der Tiefe 7–12 cm (Tiefe der Stechzylinderprobenahme) war der EW in beiden Umwelten bei C5 signifikant höher als bei P25 und C10 (Tab. 1). Die

Bodenbearbeitungsvariante C5 überschritt den kritischen Wert von 1,8 MPa in beiden Umwelten. Die Parameter GPV, LK und PL wiesen in beiden Umwelten bei C5 geringere Werte als bei P25 und C10 auf.

Tab. 1 Einfluss der Bodenbearbeitung auf physikalische Bodenparameter (Mai 2014) in Göttingen (Gö14) und Haardorf (Ha14) in 7–12 cm Bodentiefe. P25: Pflug 25 cm tief (n = 4), C10: Grubber 10 cm tief (n = 4), C5: Grubber 5 cm tief nach vorheriger Überrollung (n = 4), SM: Silomais, KM: Körnermais. Unterschiedliche Kleinbuchstaben zeigen signifikante Unterschiede zwischen den Bodenbearbeitungsverfahren über beide Umwelten. Unterschiedliche Großbuchstaben zeigen Unterschiede zwischen den Umwelten (LSD-Test, p < 0,05).

Umwelt	Vorfrucht & Bodenbearbeitung		EW [MPa]	GPV [Vol.-%]	LK [Vol.%]	PL [cm s^{-1}]
Gö14	KM+SM	P25	0,90 ± 0,37 **d**	45,99 ± 1,69 **b**	12,33 ± 2,66 **b**	5,11 ± 3,43 **c**
	KM+SM	C10	1,50 ± 0,39 **b**	42,32 ± 1,20 **d**	7,12 ± 2,21 **d**	1,54 ± 0,83 **d**
	SM	C5	1,89 ± 0,44 **a**	40,98 ± 1,18 **e**	6,14 ± 1,62 **d**	1,18 ± 1,58 **d**
			1,43 ± 0,40 **A**	43,36 ± 2,52 **B**	8,82 ± 3,55 **B**	2,79 ± 2,90 **B**
Ha14	KM+SM	P25	1,14 ± 0,51 **c**	45,93 ± 1,44 **b**	12,13 ± 2,15 **b**	8,30 ± 5,08 **b**
	KM+SM	C10	1,34 ± 0,48 **bc**	47,20 ± 0,96 **a**	14,85 ± 1,50 **a**	16,28 ± 5,08 **a**
	SM	C5	1,83 ± 0,54 **a**	43,60 ± 1,42 **c**	9,39 ± 1,91 **c**	6,37 ± 5,82 **bc**
			1,44 ± 0,51 **A**	45,97 ± 1,81 **A**	12,67 ± 2,75 **A**	11,11 ± 6,72 **A**

EW: Eindringwiderstand, GPV: Gesamtporenvolumen, LK: Luftkapazität (pF 1,8), PL: Pneumatische Leitfähigkeit (pF 1,8)

Der EW war in Gö14 und in Ha14 bei P25 am niedrigsten, wobei in Ha14 zwischen P25 und C10 kein signifikanter Unterschied bestand. Demgegenüber waren GPV, LK und PL in Gö14 bei P25 und in Ha14 bei C10 signifikant höher als bei den jeweils anderen Bodenbearbeitungsvarianten. Beim Vergleich der Umwelten waren GPV, LK und PL im Mittel über alle Varianten in Ha14 signifikant höher als in Gö14. Der mittlere EW unterschied sich zwischen beiden Umwelten nicht.

3.2 Befall mit *R. solani* an Zuckerrüben

Die maximale BS von *R. solani* an Zuckerrübe war in Gö14 mit 7 % bei P25SM im anfälligen Genotyp sehr niedrig (Abb. 2A). Alle anderen Varianten zeigten sogar nur eine mittlere BS von < 5 %. Es gab keinen Effekt von Vorfrucht/Bodenbearbeitung oder Genotyp auf die BS.

In Ha14 lag die BS für den anfälligen Genotyp im Mittel bei etwa 15 % und für den resistenten Genotyp bei 5–10 % (Abb. 2B). In beiden Umwelten wurde kein Zusammenhang zwischen BS und den Bodenparametern (EW, GPV, LK und PL) festgestellt (Tab. 2).

Tab. 2 Korrelation zwischen der Befallsstärke (BS) von *Rhizoctonia solani* in % und den Bodenstrukturparametern Eindringwiderstand (EW in MPa), Gesamtporenvolumen (GPV in %), Luftkapazität (LK in %) und Pneumatische Leitfähigkeit (PL in cm s–1) in 7–12 cm Bodentiefe sowie dem Bereinigten Zuckerertrag (BZE) und den Kenngrößen der Bodenstruktur (wie zuvor) sowie der BS in den Umwelten Göttingen (Gö14) und Haardorf (Ha14). Rangkorrelationskoeffizienten nach *Spearman*, * zeigt signifikante Koeffizienten ($p < 0{,}05$).

		EW	**GPV**	**LK**	**PL**	**BS**
Gö14	**BS**	0,20	-0,06	-0,06	-0,02	- -
	BZE	-0,10	0,21	0,21	0,12	**-0,44***
Ha14	**BS**	0,10	-0,03	0,01	0,29	- -
	BZE	**-0,45***	**0,34***	0,27	-0,13	**-0,41***

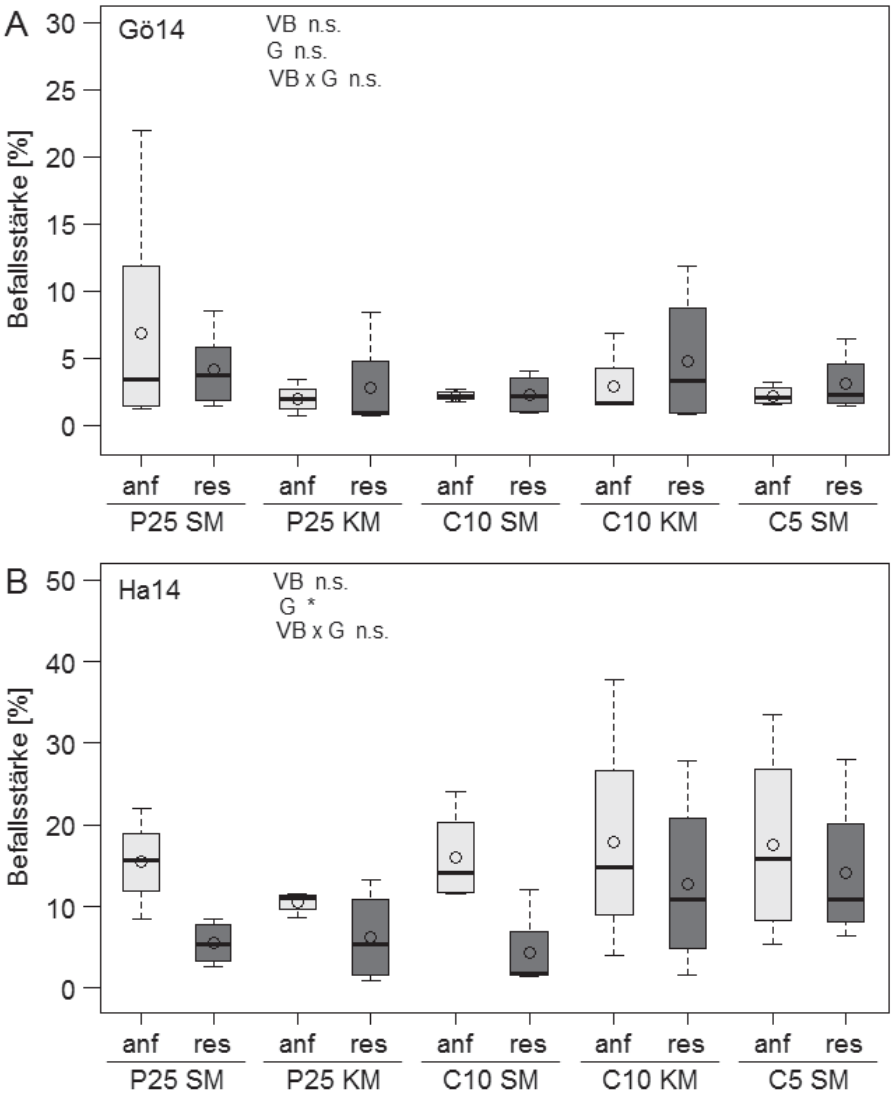

Abb. 2 Einfluss der Kombination aus Vorfrucht, Bodenbearbeitung und Genotyp auf die *Rhizoctonia solani*-Befallsstärke in % eines anfälligen (anf) und eines resistenten (res) Zuckerrüben-Genotyps in den Umwelten Göttingen (Gö14, A) und Haardorf (Ha14, B), Oktober 2014. P25: Pflug 25 cm tief (n = 4). C10: Grubber 10 cm tief (n = 4). C5: Grubber 5 cm tief nach vorheriger Überrollung (n = 4). SM: Silomais. KM: Körnermais. VB: Kombination Vorfrucht/Bodenbearbeitung. G: Genotyp. Horizontale Linie = Median, Kreis = Mittelwert, (ANOVA, $p < 0{,}05$).

In Ha14 zeigte sich ein Effekt des Genotyps auf die BS (Abb. 2B). Der anfällige Genotyp wies einen höheren Befall mit *R. solani* auf als der resistente Genotyp. Bei C10KM und C5SM war die BS tendenziell, wenn auch nicht signifikant, höher als bei den anderen Varianten. Vereinzelt wurden BS von 30–40 % beim anfälligen Genotyp und 20–30 % beim resistenten Genotyp erreicht. Ein Effekt von Vorfrucht/Bodenbearbeitung auf die BS wurde nicht festgestellt.

3.3 Bereinigter Zuckerertrag

Für die Parameter BZE und BS wurde in beiden Umwelten ein signifikant negativer Zusammenhang festgestellt (Tab. 2). Dieser war in Gö14 mit einem Bestimmtheitsmaß von 0,23 größer als in Ha14 (R^2 = 0.15, Abb. 3C). In Ha14 gab es einen signifikant negativen Zusammenhang zwischen BZE und EW (R_2 = 0,19) und einen signifikant positiven Zusammenhang zwischen BZE und GPV (R_2 = 0,11, Tab. 2, Abb. 3A, B). Für BZE und PL sowie für BZE und LK gab es in beiden Umwelten keine signifikanten Zusammenhänge (Tab. 2).

Die Kombination Vorfrucht/Bodenbearbeitung hatte in Gö14 einen signifikanten Einfluss auf den BZE (Abb. 4A). Dieser war bei C10KM gegenüber den anderen Varianten signifikant vermindert. Darüber hinaus erzielte der anfällige Genotyp einen signifikant höheren BZE als der resistente Genotyp. Die Interaktion von Vorfrucht/Bodenbearbeitung und Genotyp auf den BZE war nicht signifikant.

In Ha14 war der BZE bei C10KM und C5SM gegenüber den anderen Varianten signifikant vermindert (Abb. 4B). Ein Genotypeffekt oder ein Effekt der Interaktion Vorfrucht/Bodenbearbeitung und Genotyp war nicht vorhanden. Der insgesamt höchste BZE wurde in Ha14 bei C10SM beim resistenten Genotyp gemessen.

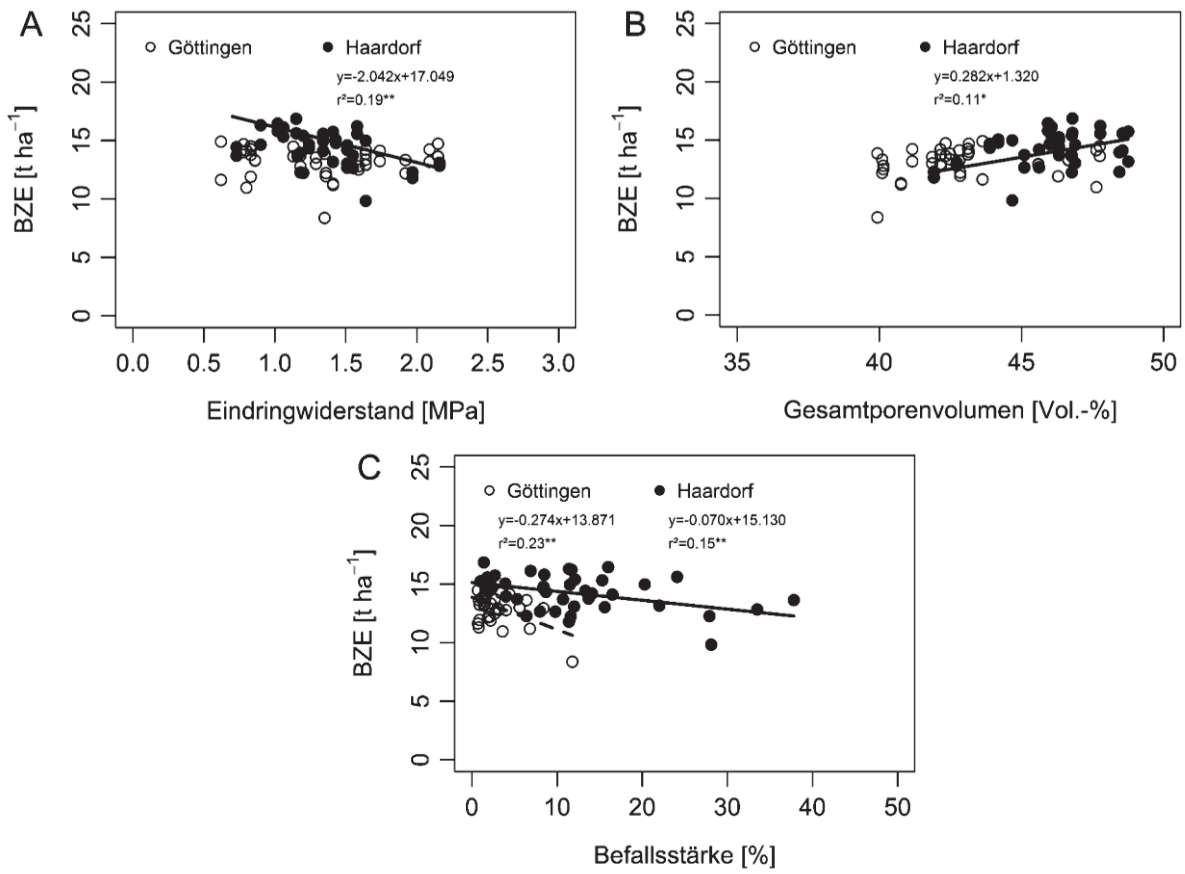

Abb. 3 Zusammenhang zwischen Bereinigtem Zuckerertrag (BZE in t ha–1) und Eindringwiderstand in MPa (A), Gesamtporenvolumen in Vol.-% (B) und Befallsstärke in % (C) (beide 7–12 cm Bodentiefe) in den Umwelten Göttingen (Gö14) und Haardorf (Ha14) (n = 40). Signifikante Zusammenhänge (p < 0,05) wurden mit linearen Modellen angepasst.

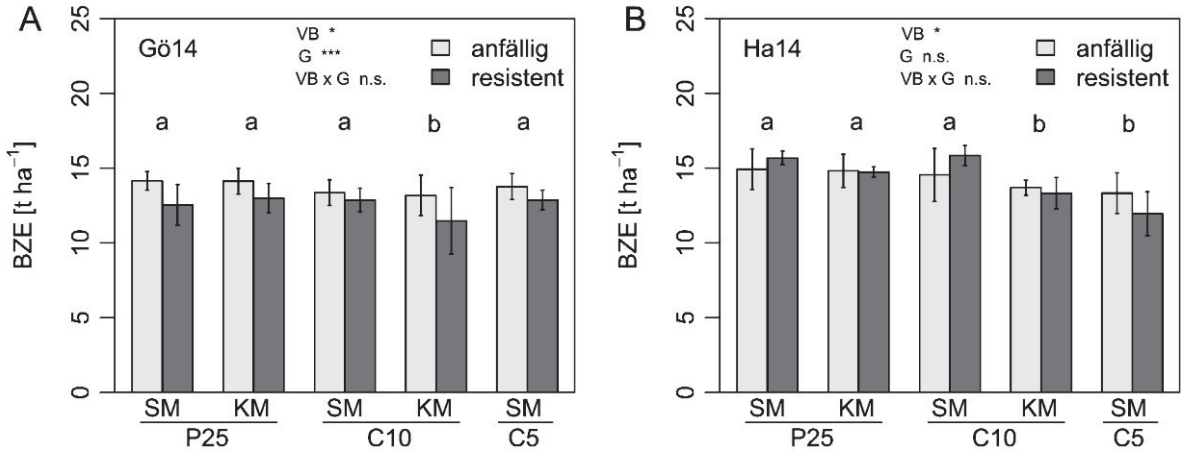

Abb. 4 Einfluss der Kombination aus Vorfrucht, Bodenbearbeitung und Genotyp auf den Bereinigten Zuckerertrag (BZE in t ha^{-1}) eines *Rhizoctonia solani*-anfälligen und -resistenten Zuckerrüben-Genotyps in den Umwelten Göttingen (Gö14) (A) und Haardorf (Ha14) (B), Oktober 2014. P25: Pflug 25 cm tief (n = 4). C10: Grubber 10 cm tief (n = 4). C5: Grubber 5 cm tief nach vorheriger Überrollung (n = 4). SM: Silomais. KM: Körnermais. VB: Kombination Vorfrucht/Bodenbearbeitung. G: Genotyp. Dargestellt sind Mittelwerte mit Standardabweichung. Unterschiedliche Buchstaben zeigen signifikante Unterschiede zwischen VB (LSD-Test, p < 0,05).

4 Diskussion

Im Rahmen dieser Arbeit wurde der Einfluss spezifischer Kenngrößen der Bodenstruktur auf den Befall mit *R. solani* an Zuckerrüben untersucht. Für diese ist bislang nicht eindeutig nachgewiesen, dass sie einen Einfluss auf den Ausbruch der Späten Rübenfäule und die Ausprägung der BS aufweisen.

Hinsichtlich der Befallsstärke wurde ein Standorteffekt zwischen Ha14 (natürlich vorkommender Befall mit *R. solani*) und Gö14 (kein natürlich vorkommender Befall mit *R. solani*) deutlich. In Ha14 war die BS höher als in Gö14, auch wenn sich das BS-Niveau im unteren Bereich der Boniturskala bewegte. Die mittlere BS in Ha14 lag mit etwa 10 % Befall beim resistenten Genotyp signifikant niedriger als beim anfälligen Genotyp mit 15–20 % (Abb. 2B). Demgegenüber war die mittlere BS in Gö14 mit ca. 5 % sogar so niedrig, dass kein Einfluss des Genotyps auf die BS zu verzeichnen war (Abb. 2A). *Buhre* et al. (2009) beobachteten in Mais-Mais-Zuckerrüben-Fruchtfolgen eine BS von etwa 1–5 % bei einem resistenten und eine signifikant höhere BS von etwa 10 % bei einem anfälligen Genotyp. *Kluth* und *Varrelmann* (2010) fanden deutlich höhere BS von bis zu 75 % bei einem *R. solani* anfälligen Genotyp nach dem Anbau von Mais als Vorfrucht. Da in Ha14 bei einer mittleren BS von 15–20 % ein Genotypeffekt auftrat, kann vermutet werden, dass der ausgebliebene Effekt des Genotyps in Gö14 durch das sehr geringe Befallsniveau bedingt war. Demgegenüber war der Einfluss des Genotyps auf den BZE sehr unterschiedlich zwischen den Umwelten. In Ha14 war der BZE durch den Genotyp unbeeinflusst (Abb. 4B), während in Gö14 der resistente Genotyp in allen Kombinationen aus Vorfrucht und Bodenbearbeitung einen geringeren BZE erzielte als der anfällige Genotyp (Abb. 4A). Ursache dafür war vermutlich die aus Sortenprüfungen (Bundessortenamt, 2014) und vorangegangenen Studien von *Buddemeyer* und *Märländer* (2005) sowie *Buhre* et al. (2009) bekannte höhere Ertragsleistung von anfälligen Genotypen gegenüber resistenten Genotypen bei Nichtbefall mit *R. solani*.

Die unterschiedlichen Bodenbearbeitungsvarianten führten in der vorliegenden Studie zu einer unterschiedlichen Ausprägung der Bodenstruktur. In beiden Umwelten nahm der EW in 7–12 cm Tiefe in der Reihenfolge P25 < C10 < C5 zu (Tab. 1). Gleichgerichtet reagierten auch die anderen Bodenparameter: GPV, LK und PL waren in beiden Umwelten jeweils in der Bodenbearbeitungsvariante am höchsten, in der der geringste EW gemessen wurde (Gö14: P25, Ha14: C10 und P25 [EW nicht signifikant unterschiedlich], Tab. 1). Allerdings gab es zwischen der BS und den Bodenparametern EW, GPV, LK und PL in beiden Umwelten keine signifikanten Zusammenhänge (Tab. 2). Nicht auszuschließen ist jedoch, dass die untersuchten Bodenparameter unter anderen Umweltbedingungen (Temperatur, Bodenfeuchte) einen Zusammenhang mit der BS aufweisen. Auch *Kühn* et al. (2009) konnten für die Lagerungsdichte, die Bodentextur und andere abiotische Bodenparameter keinen Zusammenhang zum Befall mit *R. solani* im Feld herstellen. *Otten* et al. (2004) fanden heraus, dass sich in rezenten Wurzelkanälen, die von einer dichter gelagerten Bodenmatrix umgeben sind, dichtere Pilzkolonien ausbilden können, die für einen intensiveren Wirtskontakt sorgen und den Befall mit *R. solani* erhöhen können. Außerdem ist aus Laborversuchen von *Otten* et al. (2001) bekannt, dass ein größerer Makroporenanteil zu einer stärkeren räumlichen Ausbreitung der Hyphen von *R. solani* führt. Dadurch können zwar bislang befallsfreie Feldareale erschlossen werden, die Gefahr von Primärinfektionen ist jedoch infolge der geringeren Inokulumdichte vermindert. Vor diesem Hintergrund lässt sich zusammenfassend sagen, dass die erzielte Differenzierung der bodenstrukturellen Parameter zwischen den einzelnen Versuchsvarianten entweder das Auftreten des Pilzes und dessen Befallsstärke an Zuckerrüben nicht beeinflusst oder nicht groß genug war, um einen Zusammenhang zwischen BS und den Bodenparametern sichtbar werden zu lassen. Darüber hinaus könnte der Befall mit *R. solani* aber auch zu gering gewesen sein, um Zusammenhänge zwischen BS und den Bodenparametern anzuzeigen.

Übereinstimmend mit dem nicht nachweisbaren Zusammenhang zwischen den erfassten Bodenstrukturparametern und der BS hatte die Kombination Vorfrucht/Bodenbearbeitung in keiner der beiden Umwelten einen signifikanten Einfluss auf die BS. In Ha14 war die BS in den Varianten C10KM und C5SM tendenziell höher als in den anderen Varianten und zeigte eine größere Streuung der Daten. Vereinzelt kam es zu einer BS von bis zu 40 % (Abb. 2B). Der höhere EW bei C10 und C5 verglichen mit P25 (Tab. 1) könnte ein Hinweis auf einen Einfluss der Bodendichte auf die BS von *R. solani* sein. Allerdings war diese Differenzierung bei C10SM nicht angedeutet. *Rovira* (1986) und *Cook* (2001) fanden, dass eine reduzierte Bodenbearbeitung bei Weizen und Gerste für einen höheren Befall mit *Rhizoctonia*, *Phytium* und *Fusarium* sorgte. *Pumphrey* et al. (1987) schlussfolgerten, dass eine wendende Bodenbearbeitung durch das vollständige Einarbeiten von Ernteresten und das Zerstören des Myzelnetzwerkes im Boden den Befall mit *R. solani* von Weizen verminderte. *Buddemeyer* und *Märländer* (2004) fanden bei einer Bodenverdichtung einen signifikant höheren Befall mit *R. solani* an Zuckerrüben verglichen mit einer nicht verdichteten Kontrolle. Allerdings wurde in diesen Untersuchungen keine Quantifizierung der Bodenstruktur vorgenommen. Die Ergebnisse dieser Arbeit könnten ebenfalls einen Hinweis auf eine höhere Krankheitsgefahr und höhere BS bei reduzierter Bodenbearbeitung und Bodenverdichtung gegenüber mit dem Pflug bearbeiteten Varianten geben, wobei für eine sichere Aussage weitere Ergebnisse aus dem zweiten Versuchsjahr abgewartet werden müssen.

Der Zusammenhang zwischen BS und BZE war in beiden Umwelten schwach, aber signifikant negativ (Tab. 2). Ein Zusammenhang zwischen BS und BZE wurde auch von *Buddemeyer* und *Märländer* (2004) und *Buhre* et al. (2007) beschrieben, die zeigten, dass der BZE bei anfälligen Genotypen mit zunehmender BS stärker abnahm als bei resistenten Genotypen. In Ha14 waren BZE und EW negativ und BZE und GPV positiv korreliert. Auch *Koch* (2009) fand in Versuchen mit langjährig differenzierter Bodenbearbeitung und dreifacher Überrollung des Bodens Zusammenhänge zwischen EW bzw. LK und BZE.

5 Schlussfolgerungen

In der Umwelt Ha14 war der Befall mit *R. solani* beim anfälligen Genotyp signifikant höher als beim resistenten Genotyp, während in Gö14 das Befallsniveau so niedrig war, dass kein Genotypeffekt auftrat. Die Kombination aus Vorfrucht/Bodenbearbeitung hatte in beiden Umwelten keine Wirkung auf die BS. Darüber hinaus wurden keine Zusammenhänge zwischen den Bodenparametern EW, GPV, LK und PL und der BS gefunden. Es deutete sich jedoch an, dass eine reduzierte Bodenbearbeitung möglicherweise zu einem höheren Befall mit *R. solani* an Zuckerrüben führt als eine konventionelle Pflugbearbeitung und die Krankheitsgefahr sowie die BS negativ beeinflusst. Um die Frage nach dem Einfluss spezifischer Bodenkenngrößen auf den Befall mit *R. solani* abschließend beantworten zu können, sind weitere Versuche mit einer deutlichen Differenzierung in der BS erforderlich.

In Gö14 war der BZE beim resistenten gegenüber dem anfälligen Genotyp signifikant vermindert. Ursächlich dafür war vermutlich die geringere Ertragsleistung des resistenten Genotyps bei Nichtbefall. In Ha14 mit höherer BS war der Ertrag beider Genotypen gleich. Möglicherweise war die BS nicht hoch genug, um einen Minderertrag des anfälligen Genotyps zu verursachen.

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3. Manuscript II

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Effect of Sugar Beet Variety and Nonhost Plant on *Rhizoctonia solani* AG2-2IIIB Soil Inoculum Potential Measured in Soil DNA Extracts

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Abstract

A direct soil DNA extraction method from soil samples (250 g) was applied for detection of the soil-borne sugar beet infecting pathogen *Rhizoctonia solani* anastomosis group (AG) 2-2IIIB using a newly developed real-time PCR assay that showed specificity to AG2-2IIIB when tested against various *R. solani* AGs. The assay showed a good relation between cycle threshold and amount of AG2-2IIIB sclerotia, detected in three spiked field soils and was also able to detect the pathogen in naturally infested field soil samples. A field trial was conducted to quantify *R. solani* AG2-2IIIB soil inoculum potential (IP) before and after growing a susceptible and a resistant sugar beet variety as well as after subsequent growth of an expected non-host winter rye. Plants of the susceptible sugar beet variety displayed a higher disease severity. More a six-fold increase of the *R. solani* AG2-2IIIB soil IP was observed in contrast to the resistant variety that resulted in a constant IP. Growing winter rye significantly reduced soil IP to the initial level at sowing. Further research is required to better understand the interaction between disease occurrence and soil IP as well as the environmental influence on IP development.

Additional keywords: resistance, *Rhizoctonia* crown and root rot

Introduction

The soil-borne fungus *Rhizoctonia solani* Kühn is an ubiquitous plant pathogen that affects many crops causing severe damage and yield losses worldwide as reviewed by Anees et al. (2010a). *Rhizoctonia solani* is known to represent a species complex of different anastomosis groups (AG) and further sub-groups (Ogoshi 1987). The fungus is the causal agent of the Rhizoctonia crown and root rot of sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) with AG2-2IIIB isolates identified to be the most occurring and aggressive sub-group for sugar beet (Engelkes

and Windels 1996; Bolton et al. 2010; Strausbaugh et al. 2011), however, differences in pathogenicity between isolates can be observed (Herr and Roberts 1980; O'Sullivan and Kavanagh 1991). Rhizoctonia crown and root rot disease is of increasing economic importance in European growing areas and infests about 36,000 ha each year (Garcia et al. 2001). Enormous plant losses and yield decline due to *R. solani* infestation can be observed not only in sugar beet growing areas in Europe but also in several parts of the United States of America (USA), e.g. Minnesota and North Dakota (Windels and Nabben 1989), Idaho and Oregon (Strausbaugh et al. 2011) as well as Nebraska, Colorado and Michigan (Ruppel and Hecker 1983; Kirk et al. 2008).

Like other soil-borne pathogens, *R. solani* occurs in patches due to its heterogeneous spatial distribution in the field (Herr 1996). Rotational crops such as maize, sorghum, alfalfa and different bean species, are known to be hosts of *R. solani* AG2-2IIIB and can increase disease severity in subsequently grown sugar beet (Rush and Winter 1990, Engelkes and Windels 1996, Buhre et al. 2009; Kluth and Varrelmann 2010). Integrated control strategies, including cultivation of non-host plants as rotational crops as well as tillage practices avoiding soil compaction, were shown to be appropriate to decrease disease incidence and severity (Buddemeyer and Märländer 2004; Buhre et al. 2009). In contrast to the USA, no fungicides are registered for Rhizoctonia crown and root rot management in Germany. If host plants are cultivated, less susceptible varieties with quantitative resistance traits are the most suitable measure to avoid a high disease severity and yield loss as shown by Buddemeyer and Märländer (2005) for a susceptible and a tolerant sugar beet variety.

Factors like chemical and physical soil properties, the soil microbiome or climatic conditions and their interactions are likely to affect the soil inoculum potential (IP; defined in this study as amount of target DNA per gram soil) and trigger disease outbreak as well as determine disease severity (reviewed by Anees et al. 2010a). However, these relations and interactions are not sufficiently understood, and thus, it is currently not possible to implement prediction

of disease risk in integrated control strategies. It is supposed that susceptible plant species increase the *R. solani* IP in the soil because successive production of susceptible plants in crop-rotations increase disease severity compared to alternate growth of non-hosts (Buhre et al. 2009; Kluth et al. 2010).

For the detection of *R. solani* in soils, methods like baiting with susceptible host material (Papavizas and Davey 1959), wet-sieving and direct microscopy (Boosalis and Scharen 1959) or a soil debris isolation method (Roberts and Herr 1979) have been developed earlier. Davey and Papavizas (1962) compared four methods and found indications for highly variable results between the different methods. Baiting methods are labor-intensive and give less reproducible results as shown for wet-sieving by Van Bruggen and Arneson (1986), and therefore are not suitable for large-scale field studies. Indicator plants such as *Vicia faba* L. grown in diseased field soil samples were used as a simple method to estimate the potential *R. solani* AG2-2IIIB disease severity in subsequently grown sugar beet (Dircks et al. 2014). However, pathogen biomass has to be determined for an estimation of absolute pathogen IP values. Consequently, Boine et al. (2014) developed an indirect real-time PCR assay to quantify *R. solani* AG2-2IIIB by baiting soil samples with quinoa seeds for DNA extraction, which, however, is time-consuming too.

Real-time PCR based quantification of fungal soil IP from total soil DNA extracts allows for definite identification, high accuracy and rapid detection compared to wet-sieving and baiting methods (Woodhall et al. 2012). In several studies, *R. solani* AG3 (Lees et al. 2002), AG2-1 (Zhou et al. 2014), and AG8 and AG10 (Okubara et al. 2008) were quantified from soil DNA extracts using species-specific primers in real-time PCR assays. However, in these studies, soil DNA was extracted from small soil samples of 1-10 g. This amount has to be regarded as by far too small in the background of the heterogeneous spatial distribution of *R. solani* on field scale (Martin et al. 1983; Otten et al. 2004). Thus a high number of single core samples is required to obtain one composite sample adequately representing the whole field. Next,

thorough mixing of such a composite sample is challenging because the soil usually is moist at sampling and drying might affect IP (Kinsbursky and Weinhold 1987). To alleviate such constraints, Ophel-Keller et al. (2008) and Woodhall et al. (2012) recently developed a soil DNA extraction method from large soil samples of 250-500 g combined with real-time PCR detection of soil-borne fungal pathogens.

For our study, we modified the direct DNA extraction method from 250 g soil as described by Woodhall et al. (2012): instead of using commercial DNA kits for DNA purification, we eluted DNA directly from silica particles following a silica capture based method according to Rott and Jelkmann (2001). For DNA quantification, a real-time PCR assay using new specific primers for *R. solani* AG2-2IIIB was developed. This methodology could contribute to a better estimation of the principle risk for disease occurrence, and in addition, to quantify effects of control measures on the soil-borne pathogen inoculum density. The assay was evaluated on different soils with regard to extraction efficiency and primer specificity to AG2-2IIIB. Soil samples from fields with and without Rhizoctonia crown and root rot history were tested for *R. solani* AG2-2IIIB incidence. More, the assay was applied to detect differences in *R. solani* AG2-2IIIB soil IP after growing different sugar beet varieties (susceptible, resistant) and after subsequently grown winter rye in a randomized field trial. Winter rye like wheat has never been reported to represent a host of *R. solani* AG2-2IIIB.

Materials and Methods

Fungal cultivation and DNA extraction. Fungal isolates were obtained from the American Type Culture Collection (ATCC, Manassas, USA) and the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). Isolates were cultivated *in-vitro* in potato dextrose broth (PDB, AppliChem, Germany) at 20 °C in the dark for seven days on a flat bed shaker (100 rpm). DNA extraction was performed according to the protocol of Rott and Jelkmann (2001) with the following modification. Grinding buffer was prepared with 6 M

guanidine hydrochloride instead of 4 M guanidine thiocyanate as originally described by Menzel et al. (2002). Approximately 200 mg of mycelia from the seven days old PDB cultures was ground with a pestle in liquid nitrogen prior to homogenization in grinding buffer. DNA integrity was checked by agarose gel electrophoresis (1%) and quantified spectrophotometrically (DS-11 Spectrophotometer, DeNovix, Wilmington, USA).

Production of sclerotia for spiking experiments were carried out by growing *R. solani* AG2-2IIIB (CBS101765) for 2-3 weeks on potato dextrose agar (PDA) in petri-dishes. Sclerotia were removed with a scalpel, air dried and macerated into small fragments.

Real-time PCR assay. To design *R. solani* AG2-2IIIB-specific primers for real-time PCR detection, sequences of the internal transcribed spacers (ITS) regions of different *R. solani* AGs listed in Table 1 were compared using MEGA6 software (Tamura et al. 2013). The alignment consisted of a total of 65 sequences from various *R. solani* AGs (18 AG2-2IIIB sequences) retrieved from NCBI to cover intraspecific variability. The sequences represent isolates from different geographic origin in Europe and US. AG2-2IIIB-specific regions were hand-selected from the ITS1 region to design forward (AG2-2IIIB_F: 5´-GGT TGT AGC TGG CTC CAT TAG-3´) and reverse (AG2-2IIIB_R: 5´-G TTY TCC CAT CCA TGT CTC TGC-3´) primers for real-time PCR. The forward primer AG2-2IIIB_F comprised nucleotides 16 to 36 and the reverse primer AG2-2IIIB_R nucleotides 100 to 121 of the ITS1 region of *R. solani* AG2-2IIIB isolates, respectively, resulting in an amplicon of 104 bp. Finally, primer sequences were checked against the NCBI database to prove their specificity and to identify possible unintended targets. Real-time PCR was performed in 96-well plates using the CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Munich, Germany). The iTaq™ Universal SYBR® Green Supermix (1 x) (Bio-Rad Laboratories, Munich, Germany) was used with DNA from pure fungal cultures and soil [test soils (Table 2), soil from the field trial and individual field locations] in a 20 µl reaction mix containing 1 µl extracted template DNA (25 ng) and forward and reverse primers at a final concentration of 300 nM,

respectively. PCR cycle conditions were 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s.

A standard curve of pure fungal DNA from *R. solani* AG2-2IIIB (Isolate: CBS101765, NCBI Accession number: AB054854.1) which consisted of a ten-fold dilution series with DNA amounts ranging from 520 ng to 0.52 pg was produced by plotting the cycle threshold (Ct) values against the $\log_{10}$ of the initial DNA concentration. Each sample was analyzed in triplicate and average Ct values were automatically calculated and analyzed by the Bio-Rad CFX Manager software (Bio-Rad, Laboratories, Munich, Germany) using the regression function obtained from the standard curve.

Quantification of target DNA (three technical replications) in soil samples was performed by including the pure fungal DNA standards in each PCR run. The IP (amount of *R. solani* AG2-2IIIB DNA in the soil) was expressed as ng DNA (g soil)$^{-1}$. Further, primer specificity for *R. solani* AG2-2IIIB was tested with real-time PCR and subsequent melting curve analysis including 25 ng fungal DNA of different *R. solani* isolates taken from several AGs (Tab. 1).

Table 1 Isolate code, GenBank accession number and host origin of different *Rhizoctonia solani* anastomosis groups (AG) used for the evaluation of primer specificity for *R. solani* AG2-2IIIB as well as cycle threshold (Ct) value and amplicon melting temperature (T_m) after real-time PCR. A factor of decreased sensitivity was calculated on the basis of the DNA amount using the regression function of a pure ten-fold *R. solani* AG2-2IIIB dilution series.

AG	Isolate code	GenBank accession number	Original host	Ct value	Factor of decreased sensitivity compared to AG2-2IIIB	Amp-licon T_m [°C]
AG1-1A	101759†	AB000017.1	*Oryza sativa*	-	-	-
AG2-1	101763†	DQ279000	*Pisum sativum*	-	-	-
AG2-2IIIB	101765 †	AB054854.1	*Scirpus lacustris*	17.32	-	78
AG2-2IV	101767 †	AB911323.1	*Beta vulgaris ssp. vulgaris*	20.07	6	78
AG2-2LP	RGR-38§	AB054869	*Cynodon dactylon*	20.08	6	78
AG2-3	101769 †	FJ435110	*Glycine max*	-	-	-
AG2-t	207.97 †	TCU57744	*Tulipa L.*	-	-	-
AG3	363.82†	EF532825	*Solanum tuberosum*	35.4	-	79.5
AG4-HGI	101775†	JX988994.1	*Arachis hypogaea*	-	-	-
AG4-HGII	76127‡	AB000033	*Beta vulgaris ssp. vulgaris*	-	-	-
AG5	76128‡	FJ435115	*Glycine max*	-	-	-
AG6	101779†	AF153782	soil	37.3	-	79.5
AG9	101783†	FJ435118	soil	-	-	-
AG10	971.96†	FJ435119	*Hordeum vulgare L.*	-	-	-
AG11	974.96†	FJ435120	*Lupinus angustifolius*	-	-	-
AG11	973.96†	DQ278967	*Lupinus angustifolius*	-	-	-
AG12	MYA-982‡	AF153806.1	Leaf litter	-	-	-
AG13	MYA-984‡	AB275641	*Gossypium hirsutum*	-	-	-

Soil DNA extraction. Soil samples of 250 g were used for total soil DNA extraction according to the method of Woodhall et al. (2012) with some modifications: 250 g soil (air-dried for 24 h) was mixed with 4 ml Antifoam B emulsion (Sigma Aldrich: S5631, Munich, Germany), 500 ml grinding buffer (0.1 M sodium phosphate buffer pH 8, 2 % CTAB) and 15 steel ball bearings (diameter: 20 mm) and thoroughly shaken for 4 min in a Merris Minimix Autopaint Shaker (Merris, Tuam, Ireland). A 50 ml sub-sample was centrifuged at 5,000 xg for 5 min and 20 ml of the resulting supernatant was mixed with 2 ml of 5 M potassium

acetate and incubated on ice for 10 min. The incubation step was followed by a 7 min centrifugation at 10,500 x g. Subsequently, the supernatant was transferred to a new 50 ml tube and 15 ml isopropanol and 800 µl silicon dioxide suspension was added. The mixture was incubated on a flat bed shaker at 100 rpm for 10 min followed by 7 min centrifugation at 10,500 x g. The resulting supernatant was discarded and pelleted silica particles remained.

For further purification of extracted soil DNA we used a modified silica capture method according to Rott and Jelkmann (2001) as subsequently described instead of the commercial silica-column based DNA extraction kit used by Woodhall et al. (2012): the pelleted silica particles were dissolved in 1 ml washing buffer, containing 10 mM TRIS-HCl, 0.5 mM EDTA, 50 mM NaCl and 50 % EtOH (pH 7.5), and transferred into a 2 ml reaction tube. In total, the pellet was thoroughly washed two-times in 1 ml washing buffer by vortexing and subsequent centrifugation at 6,000 xg for 1 min, respectively. The silica pellet was dried under vacuum (SpeedVac, Thermo Fisher Scientific, Braunschweig, Germany) at 45 °C for 5 min after the second washing step. Subsequently, the pellet was eluted in 300 µl HPLC-grade H_2O by vortexing and incubated for 10 min at 70 °C in a shaking incubator at 100 rpm. Silica particles were separated by centrifugation for 3 min at 13,400 xg and 250 µl of the resulting supernatant was stored at -20 °C for further real-time PCR analysis. Finally, DNA yield and integrity was checked by agarose gel electrophoresis (1 %, 120 V, 35 min) and DNA concentration was quantified spectrophotometrically (DS-11 Spectrophotometer, DeNovix, Wilmington, USA).

Assay evaluation. *Rhizoctonia solani* DNA detection efficiency and DNA extraction efficiency from soil was evaluated by spiking 250 g of three test soils (Tab. 2) with 0.5 mg to 124.5 mg of purified *R. solani* AG2-2IIIB sclerotia. Test soils were taken from fields with no history of any *R. solani*-induced disease in the past. Nine spiked samples and one unspiked control were prepared for each soil in replication for DNA extraction and following real-time PCR tests with three technical replications.To determine a potential inhibition of extracted

soil DNA on real-time PCR amplification efficiency, each *R. solani* AG2-2IIIB DNA dilution of the standard curve was mixed with 25 ng soil DNA (unspiked and free of *R. solani* AG2-2IIIB).

Table 2 Soil texture of the soils for evaluating DNA detection efficiency.

Soil	Clay < 0.002 mm [%]	Silt 0.002 - 0.063 mm [%]	Sand 0.063 - 2 mm [%]
A	16.2	73.0	10.7
B	2.1	10.5	87.4
C	2.7	16.2	81.1

Field trial design and sugar beet cultivation. A field trial was conducted near Haardorf (Germany, Lower Bavaria; 48°43'07.47" N 12°59'07.98" O) in 2013 to 2015. The soils of this region are naturally infested with *R. solani*. To increase and homogenize the *R. solani* AG2-2IIIB IP, the soil of the trial field (Luvisol; 19.2 % clay, 71.5 % silt, 9.2 % sand) was artificially inoculated in 2013 with 50 kg ha^{-1} of a barley inoculum to receive a homogenous soil IP. The barley inoculum was prepared as described by Kluth et al. (2010). Inoculation was performed immediately before maize (*Zea mays* L.; variety AgroLux; Agromais, Everswinkel, Germany) was sown as a susceptible rotational crop. The trial consisted of four replicates with five maize blocks each. The maize blocks differed in soil tillage and crop residue removal, which however, is not further considered here. In 2014, two sugar beet varieties were cultivated in plots within each maize block, resulting in 40 sugar beet plots (20 plots per variety) in total. A susceptible and a resistant sugar beet variety, with registration numbers ZR1988 and ZR1555 according to Bundessortenamt (2014), were grown. Each plot consisted of 12 rows of 14 m length with a row width of 0.5 m. Pelleted sugar beet seeds were sown at a 2-2.5 cm depth (March 2014) at 7 cm distance followed by manual singling to a final in-row distance of 21 cm at the 4-6 leaf stage. Weeds, pests and leaf diseases were controlled according to regional standards. After sugar beet harvest in 2014, winter rye (*Secale cereale* L.) was grown on the entire trial and harvested at the end of July 2015.

***Rhizoctonia solani* disease rating.** In October 2014, two rows (± 100 sugar beet taproots) in the center of each plot from the field trial were harvested by a trial harvester and beets were washed in the tare house. *Rhizoctonia solani* disease rating was performed on single beets. The linear rating scale ranged from 0 % (healthy taproot) to 100 % (completely mummified or dead taproot) in steps of 10 %. Sub-steps at 2 % disease severity (small black point-shaped lesions) and 5 % disease severity (first clearly visible lesion) were included for a better differentiation between non-diseased taproots and beets displaying initial symptoms. A mean value for every plot was calculated from single rating values. Values were displayed as disease severity (percentage of rotten sugar beet taproot surface).

Soil sampling. Soil samples for DNA extraction and determination of *R. solani* AG2-2IIIB incidence of different fields with and without a history of disease in the past were taken during the sugar beet vegetation periods in 2014 and 2015 from sugar beet fields and a long-term crop-rotation field trial located at Lower Saxony (no natural infestation) and fields located at Lower Bavaria (natural infestation area): All Lower Bavarian fields displayed disease symptoms during sampling. In contrast, fields located in Lower Saxony were symptom free, whereas the crop-rotation field trial had shown disease symptoms for the first time and was therefore used for soil sampling. Two composite soil samples were analyzed per field. One composite soil sample was obtained from at least 45 soil cores per field at 0-15 cm soil depth, as recommended by Ophel-Keller et al. (2008), to obtain approximately 2 kg of soil. Each 2 kg soil sample was thoroughly hand-mixed to receive a homogenous 250 g sub-sample which was stored at 4 °C prior to DNA extraction.

In addition, soil samples for DNA extraction and *R. solani* AG2-2IIIB quantification were taken from the field trial (Haardorf) at sugar beet sowing (March 2014) from the maize blocks, at harvest (October 2014) from the core rows of each variety plot and after growing winter rye (July 2015) from all former sugar beet variety plots in a 'w'-shaped configuration, respectively. One composite soil sample was obtained from at least 25 soil cores per block or

plot taken at 0-15 cm soil depth, as recommended by Ophel-Keller et al. (2008) for research plots, resulting in 20 composite soil samples per sugar beet variety for DNA extraction and quantification. Soil samples from the field trial were further processed as described above for soil samples from individual fields.

Data analysis. Statistical analysis was performed with the open-source software R (R Development Core Team 2014). Gaussian distribution was checked by a Shapiro-Wilk-test at $p < 0.05$ using the 'shapiro.test' function of R. Due to non normality, (i) differences of *R. solani* AG2-2IIIB DNA amounts between different soil sampling dates within the sugar beet varieties and (ii) differences of *R. solani* AG2-2IIIB disease severity between the susceptible and resistant variety were calculated using non-parametric Kruskal-Wallis-Test ($p < 0.05$) from the package 'agricolae' (de Mendiburu 2013).

Regressions were calculated using the 'lm' function of R with significance at $p < 0.05$. Analysis of covariance (ANCOVA) was performed to evaluate statistical differences between (i) regression lines of the pure fungal DNA with and without soil DNA (Fig. 1) and (ii) between regressions of the soil DNA extraction and detection efficiency from three different soil types (Fig. 2) with significance at $p < 0.05$ using the 'lm' function in R, respectively. A significant interaction term indicated differences in the slope of the regression lines.

Results

Method evaluation. Real-time PCR tests were performed to evaluate the efficiency and the specificity of the designed *R. solani* AG2-2IIIB-specific primers. Primers showed an efficiency of 102.5 % at a ten-fold dilution series of purified *R. solani* AG2-2IIIB DNA, obtained from *in-vitro* grown mycelium, with a detection limit of 0.00052 pg (Ct ~35) (Fig. 1). To evaluate a possible inhibition of DNA amplification efficiency, the pure fungal DNA dilution series was spiked with soil DNA and the real-time PCR was repeated.

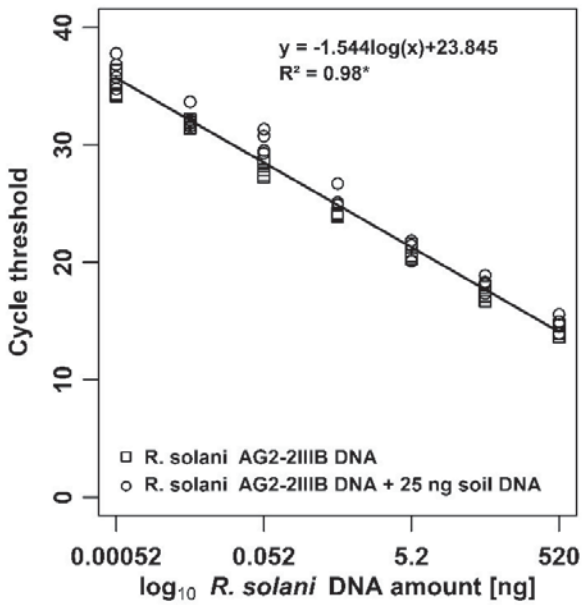

Fig. 1 Standard curve of a ten-fold *Rhizoctonia solani* AG2-2IIIB DNA dilution series ranging from 520 ng to 0.00052 ng extracted from a pure culture of *in-vitro* grown *Rhizoctonia solani* AG2-2IIIB mycelium performed without (open squares) and with (open circles) additional 25 ng soil DNA (spiked to each *R. solani* DNA dilution) for testing interference of soil DNA on amplification during real-time PCR. Points represent single values. Slopes of regression lines did not show significant differences at $p < 0.05$ (ANCOVA).

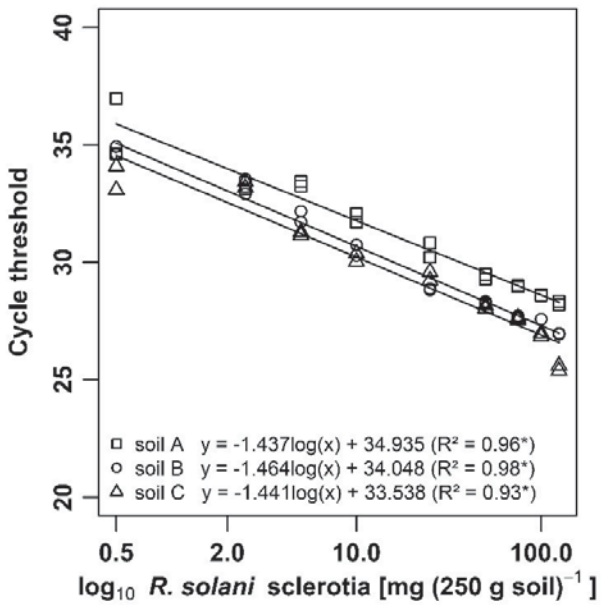

Fig. 2 DNA detection efficiency measured by cycle threshold values of different amounts of *Rhizoctonia solani* AG2-2IIIB sclerotia [mg (250 g soil)$^{-1}$] spiked into 250 g of three different test soils (A: 16.2 % clay, 73.0 % silt, 10.7 % sand; B: 2.1 % clay, 10.5 % silt, 87.4 % sand; C: 2.7 % clay, 16.2 % silt, 81.1 % sand). Amount of sclerotia spiked into the soil ranged from 0.5-124.5 mg. Points represent mean values with n = 3. Slopes of regression lines were not significantly different whereas intercepts of lines displayed significant differences at $p < 0.05$ (ANCOVA)

There was no significant difference between regressions obtained from the fungal DNA dilution series with and without spiking with extracted soil DNA and therefore, the correlation was best described by a single function (Fig. 1). The results from the cross-reaction tests (*R. solani* AG2-2IIIB-specificity) of the new primers with DNA of other *R. solani* AGs are displayed in Table 1. Primers showed DNA amplification with AG2-2IIIB, AG2-2IV and AG2-2LP. However, amplification sensitivity for AG2-2IV and AG2-2LP was six-times lower compared to AG2-2IIIB. Cycle threshold values of AG3 and AG6 were below the detection limit and amplicon melting temperature was 1.5 °C higher compared with AG2-2IIIB DNA. All other AGs did not show any amplification signal with the AG2-2IIIB primers.

Rhizoctonia solani AG2-2IIIB detection and soil DNA extraction efficiency was evaluated with three different *R. solani*-free soils (Tab. 2) spiked with *R. solani* AG2-2IIIB sclerotia. For each soil, real-time PCR with DNA extracted from the spiked soils showed a linear relationship between Ct values and the $\log_{10}$ amount of *R. solani* AG2-2IIIB sclerotia (Fig. 2). Cycle threshold values increased with decreasing amount of spiked sclerotia and ranged from 0.5-124.5 mg spiked sclerotia per 250 g soil (Ct 35-20), with mean DNA amounts of 0.04 ± 0.23 [ng DNA (g soil)$^{-1}$], respectively. The detection limit of 0.5 mg *R. solani* AG2-2IIIB sclerotia per 250 g soil was valid for the three soils. No AG2-2IIIB DNA was detected in unspiked control soil samples. There was no significant difference between the slopes of the regressions obtained for the soils, which indicates a high DNA detection efficiency in all cases with R^2 values ranging between 0.93 and 0.98. The y-intercepts of the regressions were significantly different.

***R. solani* detection in naturally infested field soils.** In all, 11 of 15 soil samples from sugar beet fields with and with no history of disease were detected positive for *R. solani* AG2-2IIIB (Table 3). *R. solani* AG2-2IIIB was not detected in all four samples from fields with no history of disease. In samples from fields with history of disease, *R. solani* AG2-2IIIB was detected in 81 % of the samples (9 of 11 samples) with a mean amount of DNA of 68.33 ng g^{-1} of soil and a range between 0.06 and 254.10 ng g^{-1} of soil.

Table 3 Incidence of *Rhizoctonia solani* AG2-2IIIB in soil samples from sugar beet fields with and with no history of Rhizoctonia crown and root rot disease with mean DNA quantity and range of positive tested samples.

Disease history	Samples[a]	Number positive (%)[b]	Mean DNA (ng g^{-1} of soil)[c]	Range (ng g^{-1} of soil)
Yes	11	9 (81)	68.33	0.06 to 254.10
No	4	0 (0)	-	-

[a] Total number of samples; [b] number of samples detected positive [c] Mean DNA quantity of positive tested samples. Detection limit: amount of sclerotia: $2 \cdot 10^{-6}$ g g^{-1} of soil ≙ DNA amount of 0.04 ng g^{-1} of soil.

Field trial. The disease rating of harvested sugar beets showed a significantly higher disease severity in case of the susceptible variety (16 % mean disease severity) compared to the resistant one (8 % mean disease severity) (Fig. 3).

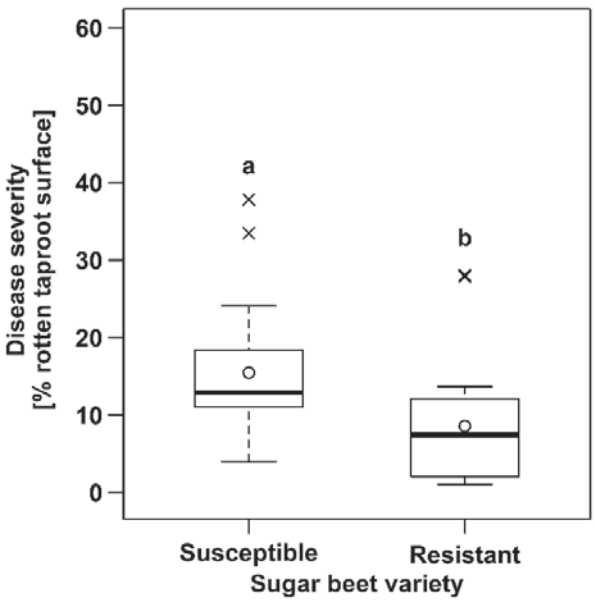

Fig. 3 *Rhizoctonia solani* disease severity of a susceptible and a resistant sugar beet variety (n = 20) after harvest at the field trial Haardorf in 2014. Black line = median, open circle = mean, cross = outlier. Different letters indicate significant differences (Kruskal-Wallis, $p < 0.05$).

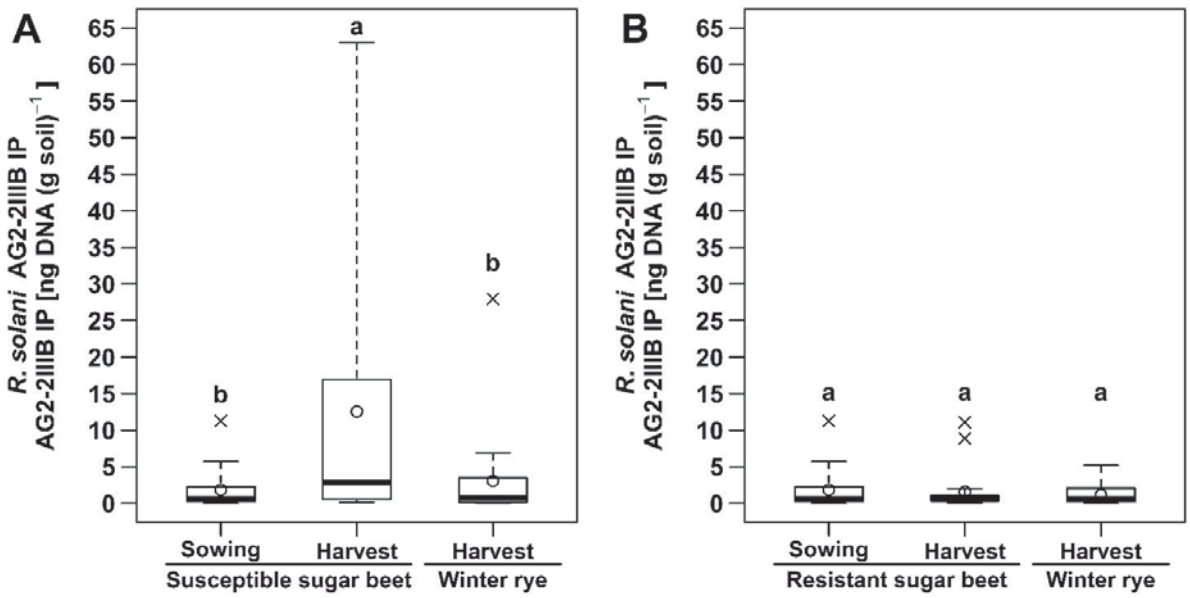

Fig. 4 *Rhizoctonia solani* AG2-2IIIB inoculum potential (IP). Soil IP was measured before sugar beet sowing (n = 20) and after sugar beet harvest (n = 20) of a susceptible (A) and a resistant (B) sugar beet variety in 2014, as well as after subsequently grown winter rye (n = 20) in 2015, respectively, at the field trial Haardorf. Black line = median, open circle = mean, cross = outlier. Different letters indicate significant differences (Kruskal-Wallis, $p < 0.05$).

The mean soil DNA amount at the field trial was 1.84 ng g^{-1} of soil for both sugar beet varieties at sugar beet sowing (Fig. 4A, B). Growing the susceptible variety caused an increase of the mean IP after sugar beet harvest by the factor of six to a DNA amount of 12.54 ng g^{-1} of soil, whereas growing the resistant variety slightly reduced the mean IP to a

DNA amount of 1.56 ng g^{-1} of soil. However, this effect was not significant. Scattering of the soil IP data was increased by growing the susceptible variety and decreased by growing the resistant variety. The growth of different sugar beet varieties resulted in a high IP variation between plots: lowest value of *R. solani* AG2-2IIIB soil IP after harvest measured in this study was a DNA amount of 0.04 ng g^{-1} of soil for the resistant variety and 0.10 ng g^{-1} of soil for the susceptible variety, and highest value was 11.09 ng g^{-1} of soil for the resistant variety and 63.03 ng g^{-1} of soil for the susceptible variety (Fig. 4A, B). Furthermore, soil IP variability was increased when the susceptible variety was grown compared to the variability obtained for the soil IP at sugar beet sowing and when the resistant variety was grown.

Growing winter rye after sugar beet revealed a significant decrease of the *R. solani* AG2-2IIIB DNA amount from 12.54 to 1.54 ng g^{-1} of soil in plots where a susceptible sugar beet variety was previously grown (Fig. 4A). In plots where a resistant sugar beet variety was previously grown the soil IP was slightly but was not significantly reduced (Fig. 4B).

Finally, *R. solani* AG2-2IIIB disease severity and IP at sugar beet sowing as well as IP after sugar beet harvest did not reveal a positive correlation for both varieties, when individual plots were analyzed (Fig. 5A, B): a higher IP at sowing did not result in a higher disease severity and a high disease severity did not necessarily result in a higher soil IP at harvest. However, the data of the individual plots of the susceptible variety was more variable at sugar beet harvest compared to data of the resistant variety that clustered mainly at low soil IP and low disease severities.

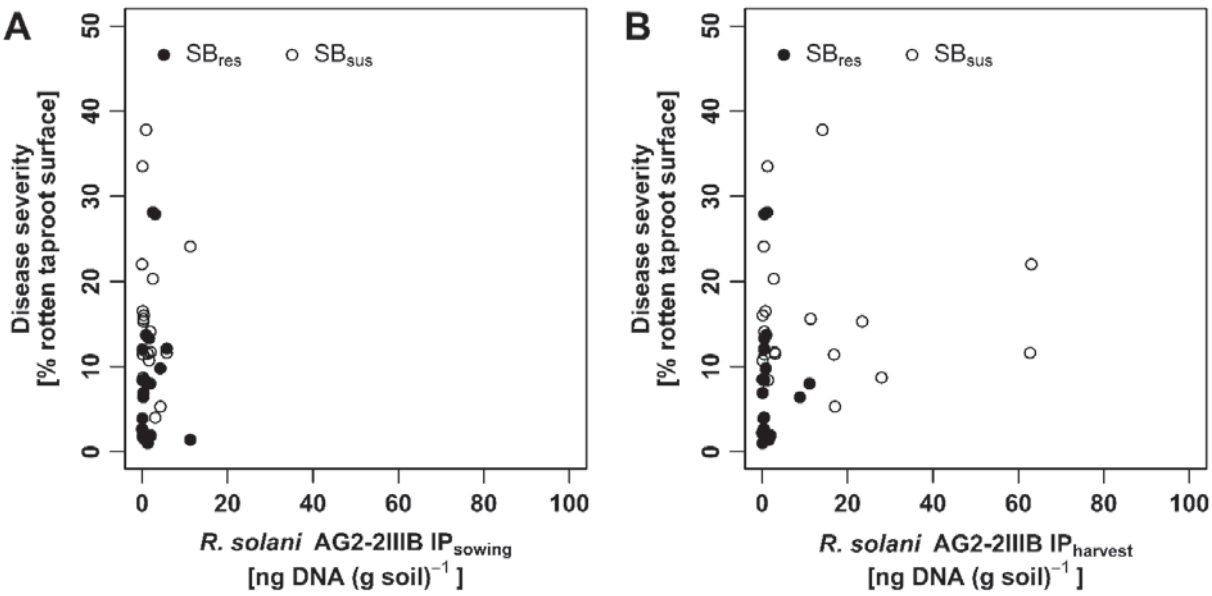

Fig. 5 Relation between the *Rhizoctonia solani* AG2-2IIIB inoculum potential in soil at (A) sugar beet sowing (IP_{sowing}) and (B) after sugar beet harvest ($IP_{harvest}$) and disease severity of the resistant (SB_{res}) and the susceptible (SB_{sus}) sugar beet variety at the field trial Haardorf in 2014. Points represent values from individual plots.

Discussion

In this study, a new reliable real-time PCR assay based on SYBR® Green following direct soil DNA extraction from 250 g soil samples for quantification of the sugar beet pathogen *R. solani* AG2-2IIIB was developed. Accurate and reliable quantification of *R. solani* AG2-2IIIB DNA was demonstrated with three test soils differing in their clay, silt and sand content. Soil DNA extraction efficiency was affected by soil type as indicated by differences in the y-intercepts of the regressions. As a consequence, when comparing the IP of different environments, the assay has to be calibrated for different soil types by spiking experiments as described in this study. Soil DNA did not inhibit real-time PCR amplification efficiency of pure *R. solani* AG2-2IIIB DNA, as there was no significant difference between regressions from the fungal DNA dilution series with and without soil DNA spiking. The method was able to detect *R. solani* AG2-2IIIB DNA in naturally infested field soil samples, with a detection limit of 0.5 mg sclerotia per 250 g of soil (= DNA amount of 0.04 ng g^{-1} of soil), and to distinguish whether a field was naturally infested with the pathogen or not. Further, it

was applied to detect differences in the soil IP of *R. solani* AG2-2IIIB before and after growing a susceptible and a resistant sugar beet variety in a *R. solani* field trial and after subsequently growing winter rye.

Specificity of the assay for detection of *R. solani* AG2-2IIIB was demonstrated in cross-reaction experiments with members of various *R. solani* AGs. Subgroup IV of AG2-2, also reported to be a pathogen of sugar beet (Büttner et al. 2002; Engelkes and Windels 1996), showed some cross-reaction. The unspecific detection of AG2-2LP is undesirable but not a serious issue for the assay because this AG was reported to be nonpathogenic for sugar beet and cause disease only on Asian zoysiagrass and other turfgrasses, for instance (Aoyagi et al. 1998; Hyakumachi et al. 1998). Furthermore, primer sensitivity with AG2-2IV and AG2-2LP was six-times lower compared to AG2-2IIIB. In case that a field is infected with one of these isolates, it would be detected with less sensitivity. Anastomosis group 3 and 6 were detected, however amplicons displayed Ct values below the detection limit and a higher melting temperature compared to our target AG2-2IIIB, indicating amplification of unspecific artifacts. For all other AGs no cross-reaction was observed in the assay including AG4 which is known to be pathogenic for sugar beet and causes seedling damping-off (Windels and Nabben 1989), indicating that the assay is not suitable for managing the damping off disease.

Nevertheless, it is worth mentioning that in other cropping systems outside of Germany or Europe, where other AGs may probably be more prevalent (e. g. AG2-2LP), the assay could still lead to false-positive results or erroneously low risk indications limiting the usability of the assay. Moreover, it needs to be mentioned that, although we aligned 65 *R. solani* AG2-2IIIB sequences to design the primers, there is the principal possibility that some isolates with variable ITS sequence as described by Strausbaugh et al. (2011) might not be detected with this assay with the same efficiency.

Woodhall et al. (2012, 2013) used additional commercial kit-based DNA purification following DNA elution from the silica particles for automation of the extraction process. This

step was replaced by a material cost-saving DNA purification with a modified silica capture method according to Rott and Jelkmann (2001). We directly eluted DNA from the silica particles without further kit-based washing steps. Our results show that direct DNA elution from silica did not lead to co-extraction of substances acting as PCR inhibitors (e.g. humic acids) (Ramakers et al. 2003; Schneegurt et al. 2003) resulting in similar detection sensitivity compared to published detection methods of other *R. solani* AGs. The detection limit of the assay was at a sclerotia amount of $2 \cdot 10^{-6}$ g g^{-1} of soil spiked in 250 g soil with a mean DNA amount of 0.04 ng g^{-1}. Detection limits previously reported for other *R. solani* AGs such as for AG3 sclerotia were $5 \cdot 10^{-4}$ g g^{-1} of soil in 10-60 g soil samples (Brierley et al. 2009; Lees et al. 2002) and $8 \cdot 10^{-7}$ g g^{-1} of soil in 250-g soil samples (Woodhall et al. 2013). Nevertheless there is a potential risk for false-positive results due to the possible detection of non-viable mycelia. This can only be avoided by use of unspecific baiting techniques combined with specific DNA quantification (Thornton et al., 1999; Lees et al., 2002; Boine et al., 2014). Direct sensitivity comparisons between baiting and direct soil-DNA extractions from large volumes, however, are required to identify the most suitable method.

Rhizoctonia solani AG2-2IIIB DNA was detected in naturally infested field soil samples at relatively low levels with a minimum DNA amount of 0.06 [ng DNA (g soil)$^{-1}$]. Nine out of eleven field soil samples with a *Rhizoctonia* crown and root rot history tested positive in this study, which illustrates the suitability of the assay to recognize Rhizoctonia crown and root rot. DNA concentrations of soil samples that tested positive were comparable with those found by Woodhall et al. (2012) for *Rhizoctonia solani* AG2-1, AG8 and *Sclerotium cepivorum* or Brierley et al. (2009) for *Colletotrichum coccodes* and *Rhizoctonia solani* AG3.

Applying the assay for soil IP analysis in a crop-rotation experiment revealed the effect of the sugar beet variety on the IP of *R. solani* AG2-2IIIB in the soil: the susceptible sugar beet variety showed both a significantly higher mean disease severity and a higher mean IP compared to the resistant variety at sugar beet harvest. Nevertheless, the disease severity of

10-20 % for the susceptible variety was unexpectedly low compared to Kluth et al. (2010) and Büttner et al. (2004), who observed disease severities up to 75 % for sugar beet in greenhouse and field experiments following artificial inoculation. However, it is worth mentioning that the susceptible variety increased soil IP even at low disease severities. The reasons for that are unknown. It can only be speculated if this can be explained by the higher parasitic activities or by indirect effects, e.g. root exudates, on the pathogen (Boosalis and Scharen 1959; Herr 1976; Naiki and Ui 1977). This underlines the importance of resistant sugar beet varieties in disease control, especially in areas with a potentially higher risk due to a long history of *R. solani*-caused severe and frequent disease occurrence in sugar beet. In these areas economic sugar beet cultivation without *R. solani* resistant varieties may be less profitable. Furthermore, it became apparent that the cultivation of winter rye, that has never been reported to be susceptible to *R. solani* AG2-2IIIB, after sugar beet significantly decreased the *R. solani* AG2-2IIIB soil IP left by growing the susceptible sugar beet variety, to the level which was determined before sugar beet sowing. These findings are in accordance with Buhre et al. (2009) and Kluth et al. (2010) who detected a positive effect of intercrops and crop-rotations with a high proportion of non-host plants on disease severity which could have been a result of decreased *Rhizoctonia* soil IP. This is supported by Ruppel (1985) who reported maize residues in soil mixtures to be conducive to *R. solani* AG2 (*Rhizoctonia* damping-off), whereas barley residues were not conducive, and that persistence of *R. solani* in crop residues may be dependent on the crop species. A positive effect of non-host crops on pathogen soil IP could possibly be traced to an enhanced antagonistic microbial community in the soil as described for *Rhizoctonia* damping-off of sugar beet (Kasuya et al. 2006) or *Rhizoctonia* root rot of apple (Cohen et al. 2005). To the best of our knowledge, this is the first study that demonstrates the different effect of a resistant and susceptible sugar beet variety on *R. solani* AG2-2IIIB IP in a randomized repeated field trial.

However, there was no correlation between disease severity and soil IP at sugar beet sowing and after sugar beet harvest, respectively. The soil IP obtained after growing the susceptible variety showed a high scattering between individual plots and, furthermore, soil IP variability was increased by growing the susceptible variety and decreased by growing the resistant variety compared to the variability of soil IP at sugar beet sowing. Growing winter rye decreased soil IP variability regardless of the previously grown sugar beet variety. This emphasizes the impact of other environmental factors, e.g. soil structural properties (Buddemeyer and Märländer 2004) or the soil microbiome (Anees et al. 2010b) on soil IP. Further, Ruppel et al. (1988) described *R. solani* population densities in a herbicide and crop rotation experiment with sugar beet, pinto bean, maize, and barley and concluded that changes in fungal population densities can be attributed to diverse environmental parameters, however, AG-specific detection of sugar beet pathogenic *R. solani* was not performed.

As previously concluded by Herr (1987) disease prediction will still be a challenging task in the future as we have shown that the IP of both sugar beet varieties at sowing did not necessarily represent a measure for disease severity at harvest. Prediction of disease risks were previously described by Ophel-Keller et al. (2008) for nematodes and soil-borne wheat and barley pathogens by allocating DNA levels to disease risk categories and Kernaghan et al. (2007), who related DNA concentrations of *Cylindrocarpon destructans* to a disease index of American ginseng.

In summary, it was shown that the assay described in the current study is suitable to detect *R. solani* AG2-2IIIB DNA in naturally infested field soils and therefore provides an alternative to other diagnostic methods to detect soil-borne pathogens. Furthermore, it can be concluded that increasing the *R. solani* AG2-2IIIB soil IP by growing a susceptible sugar beet variety might increase the risk of an elevated disease level in subsequently grown host crops, whereas growing a resistant sugar beet variety resulted in a constant soil IP on a lower level. However, a reduced soil IP due to the growth of a resistant sugar beet variety or a non-host crop might

decrease disease risk in subsequently grown host crops. Nevertheless, the development of the *R. solani* AG2-2IIIB soil IP within whole crop-rotations consisting of a variety of host and non-host crops is not yet examined over a long-term period. The new assay provides a suitable tool to answer these questions. Moreover, the effect of soil structural properties and other environmental factors on soil IP can be investigated. However, due to the lack of a correlation between disease severity and soil IP, further research is required to unravel these interactions on field-scale.

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4. Manuscript III

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Relationships between disease severity of *Rhizoctonia solani* in sugar beet (*Beta vulgaris* L.) and different soil structural conditions caused by tillage

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Abstract

Rhizoctonia solani (Kühn) is a soil-borne fungus causing Rhizoctonia crown and root rot of sugar beet. This study aimed to identify soil structural conditions promoting Rhizoctonia crown and root rot disease severity of two sugar beet variety types (resistant and susceptible). We hypothesized that an unfavorable soil structure increases pathogenicity of *R. solani* against sugar beet resulting in higher disease severity.

In 2013-2014 and 2014-2015 field experiments were conducted in two German sugar beet production areas with different infection pressure. Soil tillage and the amount of maize (preceding crop) residue were varied to obtain different soil structural conditions for subsequent sugar beet cultivation. Penetration resistance and additional soil physical parameters were determined from undisturbed soil cores. Correlation and regression analyses were performed to identify parameters affecting disease severity and white sugar yield.

Penetration resistance was found to have the main impact on white sugar yield and disease severity. Penetration resistance and white sugar yield showed a negative correlation at three environments (site × year), and disease severity was positively correlated with penetration resistance at two environments. If mean disease severity was above 15%, the susceptible variety had more severe symptoms than the resistant variety.

The pathogenicity of *R. solani* against sugar beet was enhanced by unfavorable soil structural conditions (increasing penetration resistance). This resulted in an increase in disease severity, especially for the susceptible variety. We assumed that the increasing penetration resistance led to denser *R. solani* colonies with a higher probability to infect the roots. Therefore, soil compaction and tillage practices with reduced loosening and mixing intensity should be avoided.

Keywords AG2-2IIIB, fungal pathogen, penetration resistance, pathogenicity

1. Introduction

Rhizoctonia crown and root rot, caused by the soil-borne fungal pathogen *Rhizoctonia solani* (Kühn), is of considerable concern in sugar beet production areas worldwide and can lead to severe yield losses, and moreover, negative effects in beet processing for sugar production (Büttner et al. 2004; Führer Ithurrart et al. 2004; Strausbaugh et al. 2011). In Europe, approximately 36,000 ha are infested with *R. solani* causing crown and root rot (Garcia et al. 2001), but the disease is also observed in the United States of America (Ruppel and Hecker 1983; Windels and Nabben 1989; Strausbaugh et al. 2011). The fungus is composed of different genetically isolated anastomosis groups (AG) and sub-groups, based on hyphal anastomosis reactions (Ogoshi 1987; Carling et al. 2002), with sub-group IIIB of AG2-2 identified to be the most aggressive and frequent sub-group infecting sugar beet (Engelkes and Windels 1996; Bolton et al. 2010).

Like other soil-borne pathogens, *R. solani* is heterogeneously distributed in fields and therefore, disease symptoms generally occur in patches (Herr 1996; MacNish 1996). Frequent cultivation of Rhizoctonia-susceptible crops such as maize, sorghum, soybean and other bean species within the rotation is known to increase disease severity of subsequently grown sugar beet (Engelkes and Windels 1996; Buhre et al. 2009; Kluth and Varrelmann 2010). Likewise, negative effects of short crop rotations have been reported for other *R. solani* host plants, e. g. potato (Gilligan et al. 1996; Larkin and Honeycutt 2006) or wheat (Schillinger and Paulitz 2006). Besides, remaining crop residues of host plants may also increase disease severity in sugar beet (Ruppel 1985; Dircks et al. 2014).

In Europe, the control of Rhizoctonia crown and root rot with fungicides is not feasible due to lack of registration of appropriate fungicides for sugar beet. For this reason, an integrated approach to control Rhizoctonia crown and root rot has to focus on other measures, such as agricultural strategies including cultivation of non-host plants within crop rotations and the

avoidance of soil compaction (Buddemeyer and Märländer 2004; Buhre et al. 2009). The most important measure to maintain a high yield on infested fields is the cultivation of resistant sugar beet varieties that can be traced back to American breeding material (Gaskill, 1968; Panella and Lewellen 2005). However, resistance against Rhizoctonia crown and root rot is only quantitative and cannot entirely inhibit infection, but slows down fungal colonization of sugar beet taproots (Ruppel 1973). Unfortunately, resistant varieties show a yield decline under non-diseased conditions (Märländer et al. 2003; Buddemeyer and Märländer 2005).

Moreover, the soil structure, the soil microbiome and other environmental factors as well as their interactions are likely to affect occurrence and disease severity of *R. solani*. For sugar beet it was shown that increasing temperatures and high soil moisture increased severity of Rhizoctonia crown and root rot (Bolton et al. 2010). However, these experiments were conducted in the greenhouse, raising the question of their relevance under field conditions. Furthermore, disease severity can be increased by unfavorable soil structural properties like compacted soil as shown for sugar beet and bean (Tu and Tan 1991; Buddemeyer and Märländer 2004, Buhre et al. 2009). The study by Kühn et al. (2009) revealed that the C/N ratio within diseased patches of sugar beet fields was higher compared to healthy parts of the field, but they found no relationships between pH, soil texture or bulk density and disease severity. In summary, there is little knowledge about the direct impact of single soil physical parameters on Rhizoctonia crown and root rot disease severity of sugar beet.

Therefore, this study aimed to identify individual soil physical parameters that affect Rhizoctonia crown and root rot disease severity under field conditions. We hypothesized that unfavorable soil structural conditions (i) diminish plant growth and decrease white sugar yield and (ii) increase the pathogenicity of *R. solani* against sugar beet resulting in an enhanced infection potential and greater disease severity. In order to differentiate the soil structural properties, (i) we grew maize as a *R. solani*-susceptible preceding crop to vary the amount of

maize residue (silage maize, grain maize) and (ii) we performed different soil tillage practices (plowing, tillage with a cultivator, shallow cultivation after soil compaction by wheel traffic) in two-factorial field experiments in two German sugar beet production areas (Lower Saxony and Lower Bavaria) in 2013-14 and 2014-15. Disease severity and white sugar yield of sugar beet were assessed for a susceptible and a resistant variety type.

2. Material and Methods

2.1 Site characteristics, experimental design and crop management

Field trials were conducted at Göttingen (Lower Saxony, 51°33'04.41" N 9°54'0.49" O) and Haardorf (Lower Bavaria, 48°43'07.47" N 12°59'07.98" O) in 2013/14 and 2014/15. Soil type, soil texture and weather conditions during the sugar beet vegetation period (April – October) as well as the amount of silage and grain maize crop residue and the disease severity of maize roots are listed in Table 1. The combination of site (Göttingen, Haardorf) and year (2014, 2015) resulted in four environments, named Gö14, Gö15, Ha14, Ha15.

Haardorf was located in an area with natural occurrence of Rhizoctonia root and crown rot, whereas at Göttingen, natural *R. solani* infestation was not observed in the past. To ensure and homogenize soil inoculum potential, the trial sites were artificially inoculated with 150 kg ha^{-1} (Göttingen) and 50 kg ha^{-1} (Haardorf) of a barley inoculum produced as described by Kluth et al. 2010:. The *R. solani* AG2-2IIIB isolate (Isolate: CBS101765, NCBI Accession number: AB054854.1, Preservation: Long-term storage at -70 °C) used for inoculum production was grown on potato dextrose agar at 20 °C in the dark for seven days on a flatbed shaker. Colonized potato dextrose agar was put into water and was macerated with a shaker. 1 kg of autoclaved barley grains were inoculated with 20 ml of the *R. solani* suspension. Afterwards, the inoculated barley grains were put in air-permeable plastic bags and incubated in the dark at 25 °C for two weeks. To ensure a homogeneous colonization the bags were shaken daily.

Table 1 Soil texture, climate conditions, irrigation, soil temperature and soil water content in 10 cm soil depth as well as silage and grain maize crop residue and disease severity of maize roots at Göttingen and Haardorf for the sugar beet growing period (April – October) in 2014 and 2015.

Site	Year	Soil type"	Soil texture#			Mean air temperature$	Precipitation$	Irrigation	Micro climate§ in 10 cm soil depth		Preceding crop		
											Crop residue		Mean disease severity of roots of silage and grain maize
			Clay	Silt	Sand				Mean soil temperature	Mean soil water content	Silage maize	Grain maize	
			[%]			[°C]	[mm]	[mm]	[°C]	[Vol.-%]	[Mg ha^{-1}]	[Mg ha^{-1}]	[% rotten root surface]
Göttingen	2014	Ut3	14.1 ± 0.7	75.5 ± 3.0	10.4 ± 2.9	14.6 ± 3.9	529	-	15.8 ± 2.8	18.8 ± 0.02	2.8 ± 0.3	10.2 ± 1.9	15 ± 9
Haardorf	2014	Ut4	19.2 ± 2.2	71.5 ± 1.5	9.2 ± 1.0	15.2 ± 4.0	378	-	17.8 ± 3.2	14.6 ± 0.03	2.2 ± 0.3	10.5 ± 3.4	35 ± 4
Göttingen	2015	Ut4	17.1 ± 0.8	75.1 ± 1.0	7.7 ± 0.9	16.3 ± 4.53	329	128	16.5 ± 2.5	22.9 ± 0.08	2.1 ± 0.3	13.5 ± 2.5	11 ± 3
Haardorf	2015	Ut4	18.1 ± 0.9	76.4 ± 2.1	5.5 ± 2.2	16.6 ± 4.9	333	60	17.5 ± 2.7	18.4 ± 0.05	2.1 ± 0.2	18.9 ± 5.7	20 ± 6

"0.0-0.3 m depth, according to Ad-hoc-AG Boden (2005); # particle size clay: < 0.002 mm, silt: 0.002-0.063 mm, sand: 0.063-2 mm

$data from Deutscher Wetterdienst (DWD); § mean of treatments plow + silage maize, cultivator + silage maize, wheel traffic + silage maize

After incubation, the colonized barley grains were air-dried for 8 to 10 days. Inoculum can be long-time stored in paper bags in the dark without loosening its pathogenicity. Before field application on the field, pathogenicity of the inoculum was tested *in vitro* on 14 to 16 weeks old *R. solani*-susceptible sugar beet plants. Inoculation was performed before maize (*Zea mays* L.) was sown as a *R. solani*-susceptible preceding crop in spring (2013 and 2014). Differentiation of soil structural properties was caused by variation of soil tillage after maize harvest in autumn (2013 and 2014) and consisted of (i) plowing to 0.25 m depth, (ii) soil mixing with a cultivator to 0.1 – 0.15 m depth and (iii) shallow tillage with a cultivator to 0.05 - 0.1 m depth after wheel traffic with heavy agricultural machinery. Wheel traffic (track-by-track) was conducted in autumn between maize harvest and tillage using a six-row self-propelled sugar beet tanker harvester (ROPA Euro-Tiger, total machine weight 55.0 Mg) at Haardorf and a tractor (Claas Arion 610, total machine weight 8.66 Mg) at Göttingen.

The combination of the factors of preceding crop and soil tillage resulted in five blocks with different cultivation methods (treatments): plow + silage maize (P+SM), plow + grain maize (P+GM), cultivator + silage maize (C+SM), cultivator + grain maize (C+GM) and wheel traffic + silage maize (W+SM). There was no treatment with shallow cultivation after wheel traffic with grain maize (W+GM) as preceding crop due to the large amount of crop residues of grain maize that could not be adequately incorporated into the soil. Field trials consisted of four replicates, each containing the five treatment blocks (tillage × preceding crop), resulting in 20 blocks in total.

Two sugar beet varieties differing in susceptibility towards Rhizoctonia crown and root rot according to Bundessortenamt (2015; susceptible: ZR1988; resistant: ZR1555; Syngenta Agro GmbH, Maintal, Germany) were grown in plots within the cultivation treatment blocks in a randomized split-plot design, resulting in 40 sugar beet plots per trial. Each plot consisted of 12 rows 14 m long with a row width of 0.45 m (Göttingen) and 0.5 m (Haardorf). Pelleted

sugar beet seeds were sown in March (2014) or April (2015), 2-2.5 cm deep with 7 cm between seeds followed by manual thinning to a final in-row distance of 21 cm at the 4-6 leaf stage. Due to long dry periods in 2015, the trials were irrigated if required (Table 1). Weeds, pests and leaf diseases were controlled according to regional standards. Soil mineral nitrogen (N) content in spring was about 50 kg N ha^{-1} at all environments (site × year) and N fertilization was 110 kg N ha^{-1} applied as calcium ammonium nitrate or urea ammonium nitrate was applied to the soil surface directly after sugar beet sowing in March (2014) and April (2015).The soil organic matter content was 2 % at all environments and the pH was 6.7 and 6.2 at Göttingen in 2014 and 2015 and 6.6 and 6.9 at Haardorf in 2014 and 2015.

2.2 Sugar beet leaf area index and soil structural parameters

Leaf area index was measured with a LAI-2200 instrument (LI-COR, Lincoln, Nebraska, USA) following the procedure described by Röver and Koch (1995). The plant canopy size was determined for each sugar beet plot once in June, July, August and September.

Soil penetration resistance was measured within each cultivation treatment block to a depth of 40 cm immediately after sugar beet sowing (March 2014, April 2015) with a penetrologger (Eijkelkamp, Giesbeek, Netherlands), equipped with a cone having a cross-sectional area of 1 cm^2 and an angle of 60°. Measurements were performed with ten internal replicates per block on a diagonal. A mean PR value was calculated for the depth of 7-12 cm.

Moreover, undisturbed soil core samples were taken after thinning of sugar beets in May to determine soil physical properties. In total, 10 soil cores each of 250 cm^3 volume (5 cm high, 8 cm in diameter) were taken vertically from 7-12 cm depth in all cultivation treatment blocks from an area located in the center of the block. The depth of 7-12 cm for soil core sampling was chosen because the majority of sclerotia and disease symptoms (lesions, rotted root tissue) can be found in the upper 15 cm of the topsoil (Naiki and Ui 1977). Afterwards, the soil cores were capillary wetted to saturation on a sand bed and then drained at a water

tension of 6.2 kPa (pF 1.8, field capacity). Subsequently, the core weight was determined. Finally, the cores were oven-dried (105 °C) to constant weight. Bulk density was derived from core dry weight and volume. Total pore volume was calculated from bulk density assuming a particle density of 2.65 g cm^{-3} (quartz). Air capacity was calculated from the difference of pore volume and field capacity.

Additionally, soil temperature and soil water content were measured hourly from April to October with 5TE sensors and logged with an Em50 data logger (Decagon, Pullman, USA). At each field trial, five sensors were installed at 10 cm depth in one P+SM, C+SM and W+SM plot resulting in 15 sensors per trial. Hourly mean values were calculated for soil temperature and soil water content.

2.3 Sugar beet yield and Rhizoctonia crown and root rot disease rating

Two rows (approximately 100 sugar beet taproots) in the center of each sugar beet plot were machine harvested in October. Sugar beets were weighed to determine root weight and further processed to produce brei samples, which were rapidly frozen at -80°C. For analysis, brei was clarified with a 0.3% aluminum sulfate solution and filtrates were used to determine the technical quality parameters (potassium , sodium and amino N) in an automatic beet laboratory system (Venema Installations, Eemshaven, NL or Anton Paar GmbH, Ostfildern-Scharnhausen, DE) as described by Hoffmann (2006). The sucrose content was measured polarimetrically. White sugar yield was calculated for each plot using the sugar beet root weight, the sugar content and the standard molasses loss according to formulas given by Märländer et al. (2003).

Disease severity rating (approximately 100 beets per plot) was performed on single beets after washing in the tare house according to a linear rating scale ranging from 0% for a healthy taproot to 100% for a completely mummified or dead taproot as described by Schulze et al.

(2016). For each sugar beet plot, a mean value was calculated from single ratings and expressed as disease severity (percentage of rotten sugar beet taproot surface).

2.4 Data evaluation and statistical analysis

Data evaluation was carried out with the software 'R' (R Core Team, 2015). Data was tested for normality by Shapiro-Wilk's test at $P < 0.05$ using the 'shapiro.test' function of 'R' and transformed to approximate normal distribution if necessary. For statistical evaluation, each combination of site and year was defined as environment. For white sugar yield and disease severity, analysis of variance (ANOVA) was performed using the 'nlme' package of 'R' (Pinheiro et al., 2013), with environment, variety,cultivation treatment and its interaction set as fixed effects and replication nested within environments set random with significance at $P \leq 0.05$. The ANOVA for penetration resistance and air capacity of all four environments was performed as abovementioned with environment, cultivation treatment and its interaction as fixed effects and replication nested within environments set random with significance at $P \leq 0.05$. For leaf area index, ANOVA was performed with treatment and variety as fixed effects and replication set random with significance at $P \leq 0.05$. When main effects or interactions were significant ($P \leq 0.05$), individual means were compared with Fisher´s LSD-test (with Bonferroni adjustment) using the function 'LSD.test' from the package 'agricolae' of 'R' (de Mendiburu, 2013). Furthermore, Pearson´s correlation coefficients were separately calculated with the 'rcorr' function of the 'Hmisc' package (Harrell, 2015) for both varieties within each environment.

All regressions were calculated using the 'lm' function of 'R' for linear models with significance at $P \leq 0.05$. Due to the fact, that correlation analysis revealed that the soil structural parameters were correlated among each other, which did not allow for a distinct separation of the effect of a single parameter from the effect of others. Thus, we decided to

choose the penetration resistance as the only explanatory variable for the regression analysis because it showed the closest correlation with the response variables disease severity and white sugar yield. In addition, soil moisture and temperature were not taken into account in the correlation analysis because they were similarly linked to the penetration resistance. Moreover, Gö14 and Ha14 were excluded from the regression analysis due to the missing correlation between disease severity and soil structural properties, and Gö15 and Ha15 were evaluated separately from each other with regard to the large difference in disease severity existing, which might have masked relationships in an overall approach.

Analysis of covariance (ANCOVA) was performed to evaluate significant differences ($P \leq 0.05$) between slopes of the regression lines of the two sugar beet varieties (Fig. 4-6) by insertion of a 'dummy variable'. A significant interaction term indicated differences in the slope of the regression lines. For Gö15 and Ha15 each, a separate ANOVA was calculated for white sugar yield and disease severity including variety as a fixed effect, using the 'lme' function of 'R' from the package 'nlme' (Pinheiro et al., 2013). In a second step, penetration resistance was introduced as a covariate and an ANCOVA was performed as mentioned above. Thereby, the variety effect was adjusted for the impact of penetration resistance.

3. Results

3.1 Soil physical parameters

Both factors, environment and cultivation treatment as well as their interaction had significant effects on penetration resistance and air capacity in 7-12 cm soil depth (Table 2). At all environments highest penetration resistance was measured in the W+SM treatment ranging from 1.8 to 2.3 MPa (Fig. 1a). In the other treatments, penetration resistance ranged from 0.8 to 1.4 MPa. At Ha14 and Gö15, penetration resistance values obtained from P+SM, P+GM,

C+SM and C+GM were not significantly different. In contrast, at Gö14, penetration resistance of C+SM and C+GM was significantly higher compared to P+SM and P+GM, whereas at Ha15 only C+SM was significantly higher compared to P+SM and P+GM.

Table 2 Analysis of variance for the effects of environment (site × year) and cultivation treatment (tillage × preceding crop) on penetration resistance and air capacity in 7-12 cm soil depth, 4 environments, 2014-2015.

	Penetration resistance		Air capacity	
	F-value	*P*-value	F-value	*P*-value
Environment (E)	10.31	< 0.0001	64.22	< 0.0001
Cultivation treatment (T)	89.08	< 0.0001	9.87	< 0.0001
E × T	7.13	< 0.0001	10.99	< 0.0001

The air capacity values showed a higher variation compared to penetration resistance values (Fig. 1b). Nevertheless, air capacity tended to react almost inversely to the treatments compared to penetration resistance. This resulted in the lowest air capacity in the W+SM treatment at all environments. In all, air capacity was generally higher at Haardorf (Ha14, Ha15) than at Göttingen (Gö14, Gö15). At Gö14, C+SM and C+GM showed similarly low air capacity compared to W+SM, while at the other environments C+SM and C+GM had comparably high (Gö15, Ha15) or even higher (Ha14) air capacity than P+SM and P+GM. The grain maize treatment had significantly higher air capacity than those with silage maize at Gö15 only, while the opposite tended to occur at Ha15.

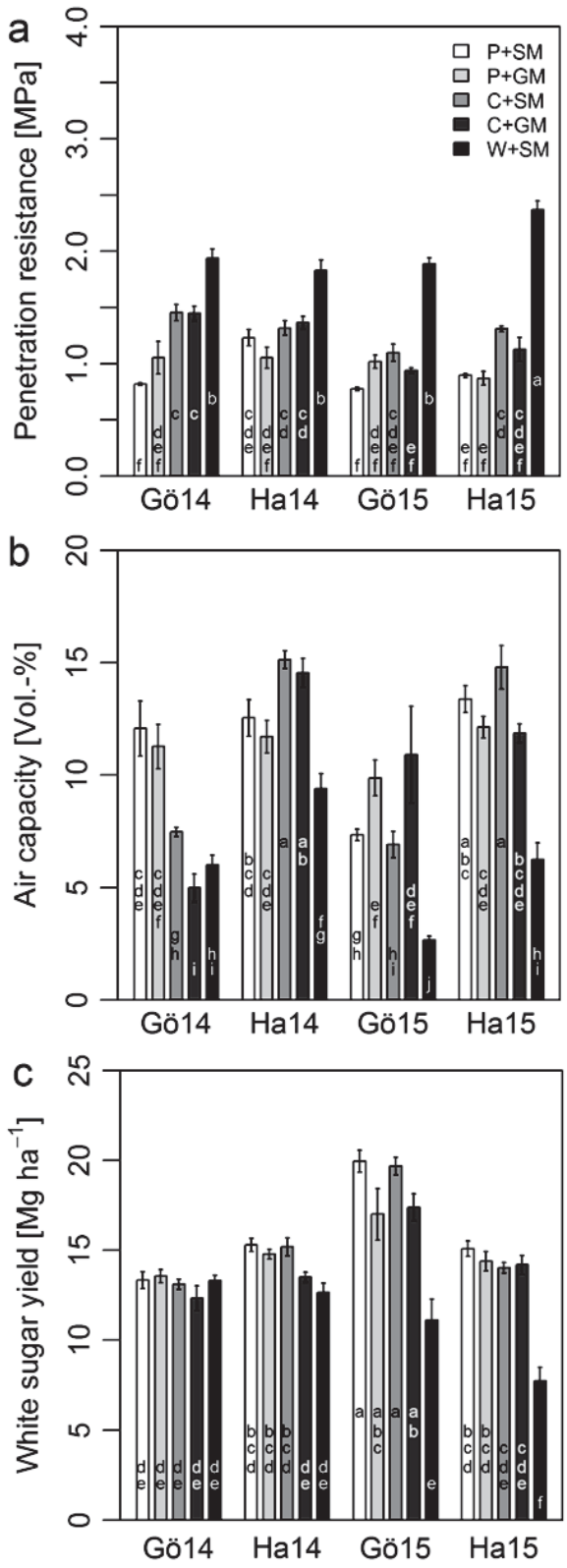

Fig. 1 Treatment effect on a) penetration resistance and b) air capacity at a soil depth of 7-12 cm, and c) white sugar yield of sugar beet at Göttingen and Haardorf in 2014 (Gö14, Ha14) and 2015 (Gö15, Ha15). Bars are means of two sugar beet varieties ± SE, n=4. Treatments: P = plow, C = cultivator and W = wheel traffic with SM = silage maize and GM = grain maize as preceding crop. Different lowercase letters indicate significant differences among treatments across environments and different uppercase letters indicate significant differences among environments ($P \leq 0.05$, LSD-test)

3.2 White sugar yield and disease severity of Rhizoctonia crown and root rot

At Gö14 and Ha14, there was no treatment effect on the leaf area index in September, while in June (Gö14), and July and August (Ha14) significant differences between treatments occurred (Fig. 2a, b). At Gö15 and Ha15 however, the leaf area index was lower at W+SM compared to the other treatments during the whole vegetation period, reaching a maximum value of 2.5-3 in September (Fig. 2c, d). In the other treatments, the leaf area index was 2-3 in June and 5-6 in September. In all, the leaf area index reached lower maximum values in Gö14 and Ha14 compared to Gö15 and Ha15.

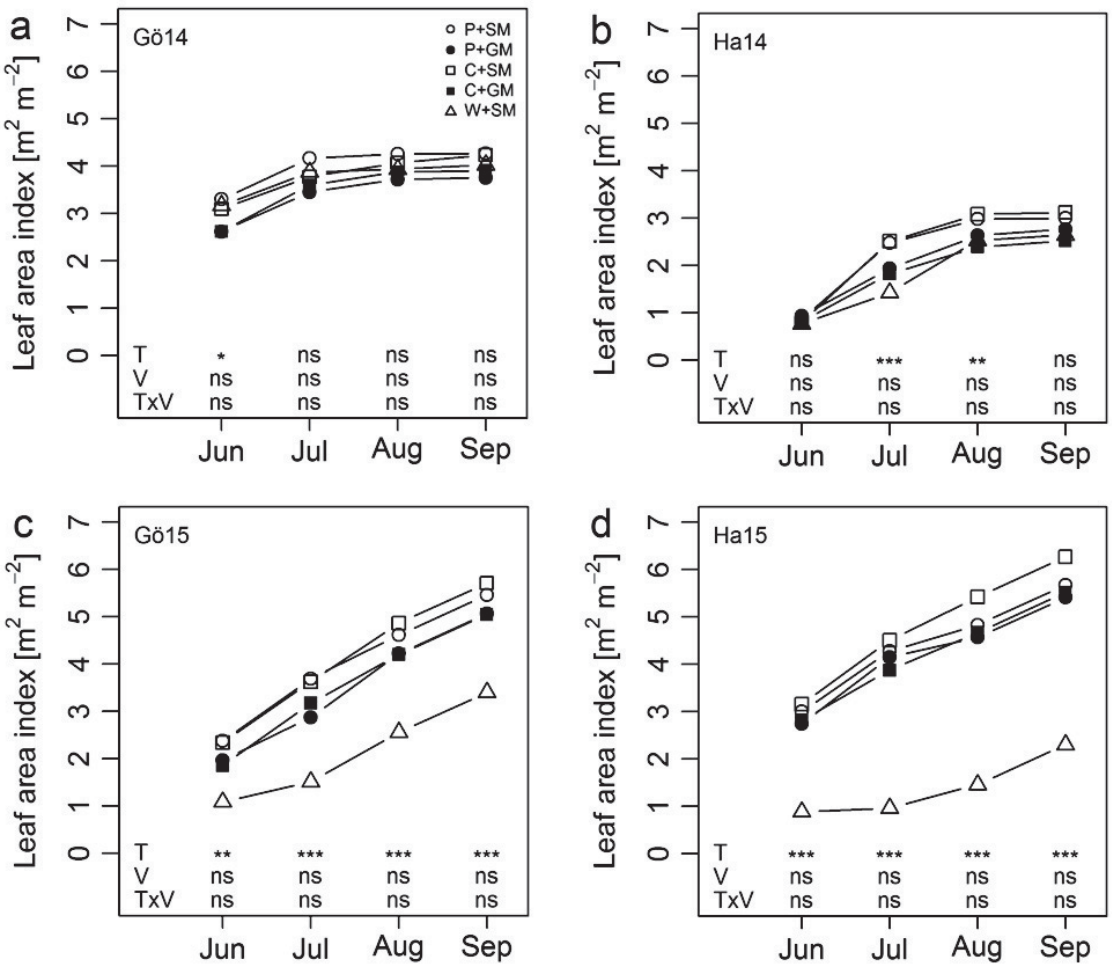

Fig. 2 Effect of cultivation treatment (tillage × treatment) on leaf area index of sugar beet at four dates during the growing season at Göttingen and Haardorf in 2014 (Gö14, Ha14) and 2015 (Gö15, Ha15); n = 4. Each sampling date was analyzed separately. P = plow, C = cultivator and W = wheel traffic with SM = silage maize and GM = grain maize as preceding crop. The level of significance is indicated by *, ** and *** at $p \leq 0.05$, 0.01 and 0.001, respectively; ns = not significant.

Variation of white sugar yield was mainly attributed to the effects of environment and cultivation treatment, and their interaction (significant at $P < 0.001$; Table 3), while the effect of the sugar beet variety was not significant. White sugar yield was highest at Gö15 with a yield of about 19 Mg ha^{-1} at P+SM and C+SM (Fig. 1c), and lowest at W+SM at Gö15 and Ha15 with a white sugar yield of 8-10 Mg ha^{-1}, whereas the white sugar yield from other treatments did not differ. At Gö14 and Ha14, treatments did not differ in white sugar yield.

Rhizoctonia crown and root rot disease severity was mainly affected by the interaction of environment and variety (significant at $P < 0.001$), while the effect of the cultivation treatment was significant at $P < 0.01$ (Table 3).

Table 3 Analysis of variance for the effects of environment (site × year), cultivation treatment (tillage × preceding crop) and sugar beet variety on white sugar yield and Rhizoctonia crown and root rot disease severity of sugar beet, 4 environments, 2014-2015.

	White sugar yield		Disease severity	
	F-value	*P*-value	F-value	*P*-value
Environment (E)	47.27	< 0.0001	48.69	< 0.0001
Variety (V)	0.02	0.8689	43.06	< 0.0001
Cultivation treatment (T)	36.61	< 0.0001	7.91	0.0023
E × V	2.53	0.0609	20.44	< 0.0001
E × T	8.57	< 0.0001	1.63	0.0928
V × T	0.58	0.6761	0.11	0.9649
E × V × T	1.02	0.4294	0.49	0.9128

Across varieties, W+SM significantly increased disease severity compared to P+SM, P+GM and C+SM, while C+GM was intermediate (Fig. 3). At Gö14 and Gö15, the two sugar beet varieties showed no differences in disease severity (Fig. 4). The mean Rhizoctonia crown and root rot disease severity was below 10% of rotten taproot surface for both the resistant and the susceptible variety. In contrast, at Ha14 and Ha15 disease severity was significantly higher in the susceptible variety (15-28%) than in the resistant variety (8-10%) with highest disease severity measured at Ha15 at all.

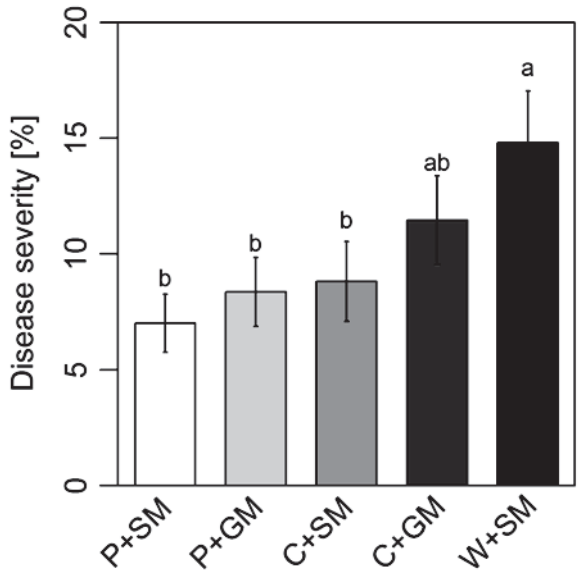

Fig. 3 Effect of cultivation treatment (tillage × preceding crop) on Rhizoctonia crown and root rot disease severity of sugar beet. Bars are means of two varieties and four environments in 2014-2015 ± SE, n=16. Treatments: P = plow, C = cultivator and W = wheel traffic with SM = silage maize and GM = grain maize as the preceding crop. Different letters indicate significant differences among treatments ($P \leq 0.05$, LSD-test).

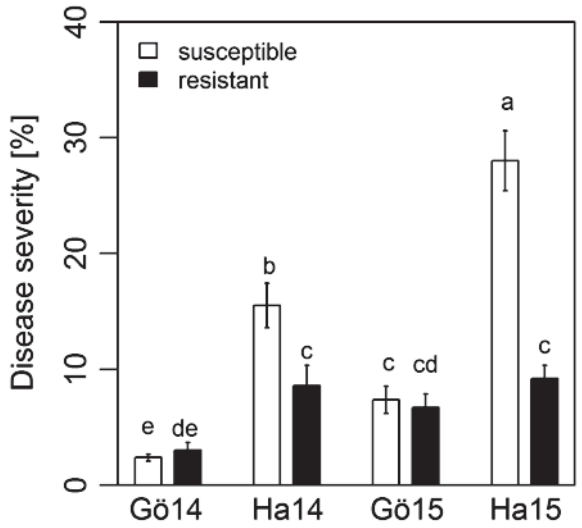

Fig. 4 Effect of the susceptible and resistant sugar beet variety on Rhizoctonia crown and root rot disease severity at Göttingen and Haardorf in 2014 (Gö14, Ha14) and 2015 (Gö15, Ha15). Bars are means ± SE, n=20. Different letters indicate significant differences between varieties across environments ($P \leq 0.05$; LSD-test).

3.3 Relationships between soil structural parameters, disease severity and white sugar yield

Pearson´s coefficients revealed a significantly negative correlation between white sugar yield and disease severity for both sugar beet varieties at all environments, except for the susceptible variety at Ha14 (Table 4).

Table 4 Pearson´s coefficients of correlation between white sugar yield (WSY) of sugar beet, Rhizoctonia crown and root rot disease severity (DS) on sugar beet taproots, penetration resistance (PR) and air capacity (AC) in 7-12 cm soil depth at Göttingen and Haardorf in 2014 (Gö14, Ha14) and 2015 (Gö15, Ha15). Significant coefficients are in bold print at $P \leq 0.05/0.01/0.001$ are marked with */**/*** (N=20).

Environment		Susceptible sugar beet variety			Resistant sugar beet variety		
		DS	PR	AC	DS	PR	AC
Gö14	WSY	**-0.52***	-0.22	0.31	**-0.49***	0.14	0.19
	DS		-0.12	-0.14		0.21	-0.22
	PR			**-0.47***			**-0.47***
Ha14	WSY	-0.11	**-0.45***	0.20	**-0.73*****	**-0.49***	0.33
	DS		0.20	-0.02		0.02	0.06
	PR			**-0.44***			**-0.44***
Gö15	WSY	**-0.83*****	**-0.79*****	0.34	**-0.85*****	**-0.76*****	**0.54***
	DS		**0.53***	-0.35		**0.55***	**-0.47***
	PR			**-0.60****			**-0.60****
Ha15	WSY	**-0.87*****	**-0.84*****	**0.77*****	**-0.62****	**-0.86*****	**0.72*****
	DS		**0.62****	**-0.52***		**0.70*****	**-0.62****
	PR			**-0.72****			**-0.72*****

The highest coefficient observed was r = -0.87 for the relationship between white sugar yield and disease severity for the susceptible variety at Ha15. At all environments, there were significant correlations among penetration resistance, air capacity and total pore volume (data for the total pore volume not shown). Furthermore, there was a significantly negative relationship between white sugar yield and penetration resistance for both varieties at Gö15, Ha14 and Ha15 with the highest coefficients observed at Ha15. At Gö14, white sugar yield

and penetration resistance showed no significant relationship. The relationship between white sugar yield and air capacity was significant at Gö15 (resistant variety) and Ha15, but the correlation coefficients were lower compared to the relation between white sugar yield and penetration resistance.

Disease severity was not significantly correlated to penetration resistance and air capacity at Gö14 and Ha14. However, at Gö15 and Ha15 the disease severity of both sugar beet varieties revealed a significantly positive relationship to penetration resistance (r = 0.53-0.70). Additionally, the disease severity of both varieties was significantly negatively correlated to the air capacity at Ha15, whereas at Gö15 only the resistant variety showed this relationship.

Due to lack of significant correlations of the disease severity with soil structural parameters at Gö14 and Ha14, linear regressions were calculated for the environments Gö15 and Ha15 only. For the relationship between disease severity and white sugar yield and between penetration resistance and white sugar yield, the slopes of the regression lines of the two varieties were not statistically different at either environment (Fig. 5a, b and 6a, b).

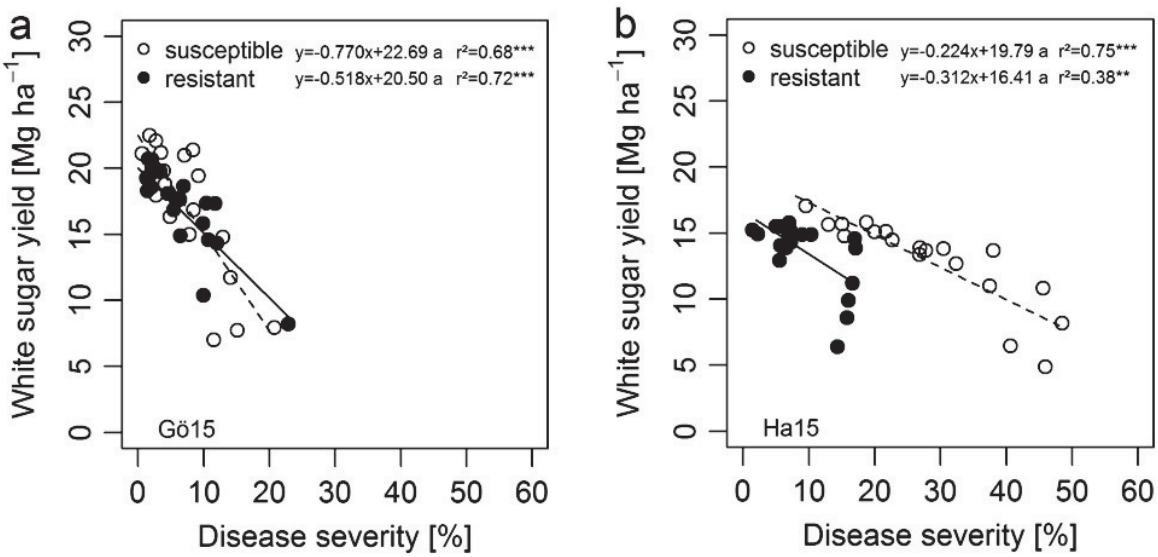

Fig.5 Relationship between Rhizoctonia crown and root rot disease severity and white sugar yield of the susceptible and the resistant sugar beet variety at a) Göttingen (Gö15) and b) Haardorf (Ha15) in 2015. Different letters indicate significant differences between the slopes of the regression lines ($P \leq 0.05$, ANCOVA). The level of significance of the slopes is indicated by *, ** and *** at $p \leq 0.05$, 0.01 and 0.001, respectively; ns = not significant.

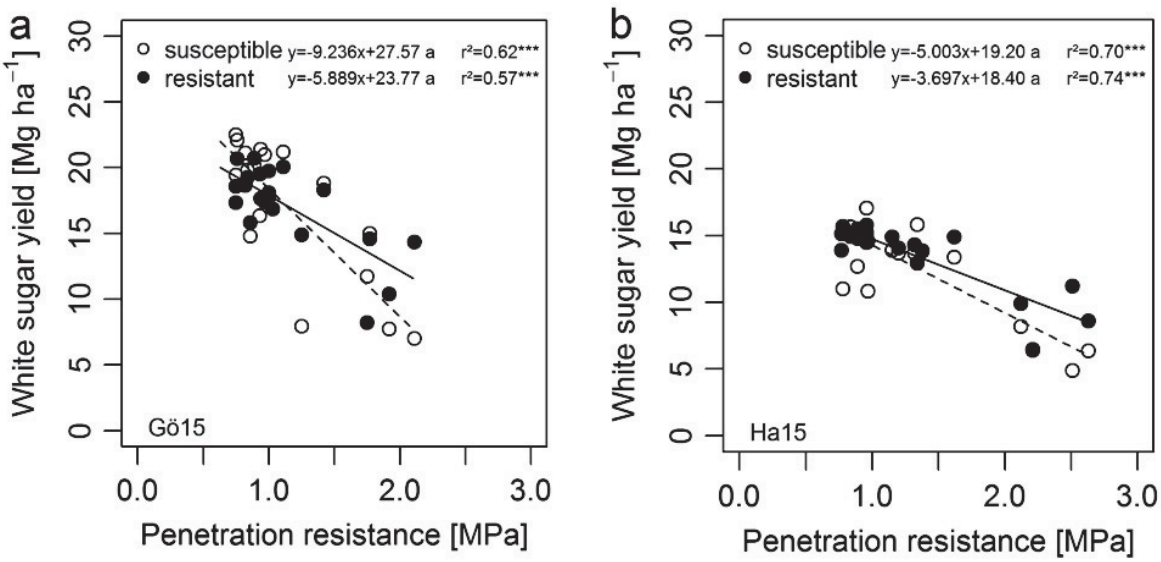

Fig. 6 Relationship between penetration resistance and white sugar yield of the susceptible and the resistant sugar beet variety at a) Göttingen (Gö15) and b) Haardorf (Ha15) in 2015. Different letters indicate significant differences between the slopes of the regression lines ($P \leq 0.05$, ANCOVA). The level of significance of the slopes is indicated by *, ** and *** at $p \leq 0.05$, 0.01 and 0.001, respectively; ns = not significant.

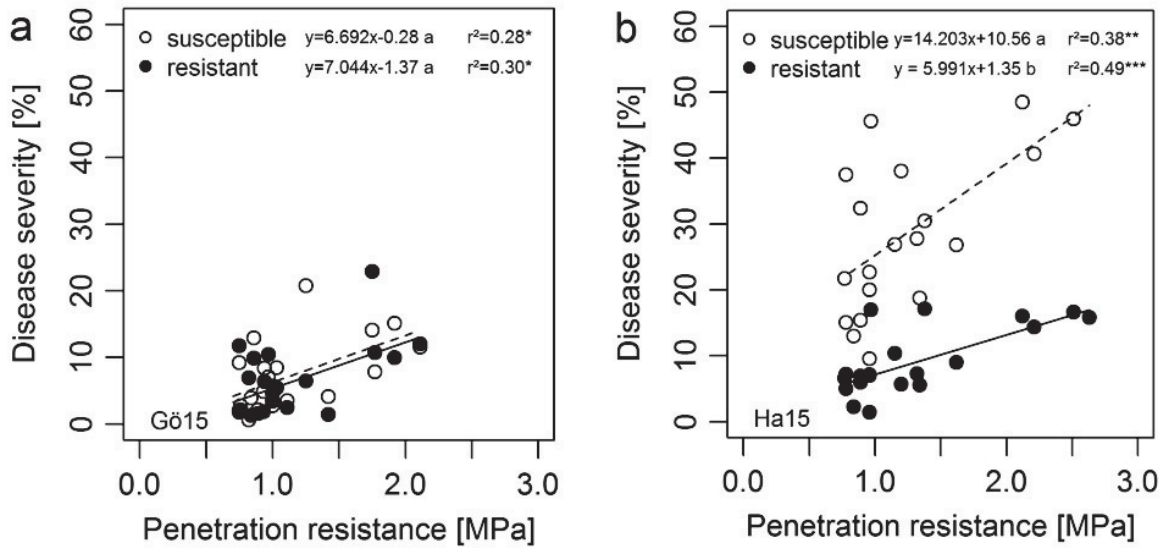

Fig. 7 Relationship between penetration resistance and Rhizoctonia crown and root rot disease severity of the susceptible and the resistant sugar beet variety at a) Göttingen (Gö15) and b) Haardorf in 2015. Different letters indicate significant differences between the slopes of the regression lines ($P \leq 0.05$, ANCOVA). The level of significance of the slopes is indicated by *, ** and *** at $p \leq 0.05$, 0.01 and 0.001, respectively; ns = not significant

Nevertheless, white sugar yield losses were more severe at Gö15 compared to Ha15, as indicated by the steeper slopes for data from Gö15 for both varieties. For the relationship between disease severity and penetration resistance the slope of the regression line was

significantly steeper for the susceptible compared to the resistant variety at Ha15 but not at Gö15 (Fig. 7a, b).

Introducing penetration resistance as covariate into a simple fixed effect model of variance and co-variance for white sugar yield and disease severity confirmed a significant effect of penetration resistance for both parameters at Gö15 and Ha15 (Table 5). The effect of the sugar beet variety without covariate was significant for the disease severity at Ha15 only, and remained significant when penetration resistance was introduced as a covariate.

Table 5 Analysis of variance for the effect of the sugar beet variety on white sugar yield and Rhizoctonia crown and root rot disease severity of sugar beet at Göttingen (Gö15) und Haardorf (Ha15) without and with penetration resistance as a covariate in 2015.

		White sugar yield		Disease severity	
Environment		F-value	*P*-value	F-value	*P*-value
Gö15	Without covariate:				
	Variety	0.000	0.9830	0.166	0.6860
	With Covariate:				
	Penetration resistance	51.024	< 0.0001	15.290	0.0004
	Variety	0.001	0.974	0.229	0.6351
Ha15	Without covariate:				
	Variety	0.858	0.3530	44.060	< 0.0001
	With Covariate:				
	Penetration resistance	87.754	< 0.0001	13.060	0.0008
	Variety	2.907	0.0966	58.050	< 0.0001

4. Discussion

Although it is supposed that the soil structure has a substantial impact on Rhizoctonia crown and root rot disease severity of sugar beet, there is little knowledge about relationships between specific soil structural parameters and disease severity under field conditions. Our study confirmed that disease severity is highly dependent on the environment and interacts with the susceptibility of the sugar beet variety grown.

At the non-naturally infested but artificially inoculated environments Gö14 and Gö15 the mean disease severity was below 10% of rotten taproot surface, and even at the naturally

infested plus inoculated environments Ha14 and Ha15 the mean disease severity was 10-30% only. Contrastingly, Büttner et al. (2004) and Kluth et al. (2010) reported a disease severity up to 75% for sugar beet in field and greenhouse experiments following artificial inoculation immediately before sugar beet sowing in spring; in our study the inoculum was incorporated before sowing of maize as the preceding crop. Moreover, at Gö14 and Gö15 disease severity did not differ between the susceptible and the resistant sugar beet variety, while it was significantly higher for the susceptible compared to the resistant variety at Ha14 and Ha15, thereby confirming previous findings of Buddemeyer and Märländer (2005) and Buhre et al. (2009) for different crop rotations.

The lack of differences in disease severity between the two varieties at Gö14 and Gö15 may be attributed to micro-climatic conditions, such as soil temperature and moisture, or/and the overall low disease severity level. Mean soil temperature during the sugar beet growing season was between 15.8 and 17.8 °C at all environments which is known to promote *R. solani* establishment (Bolton et al. 2010). The soil water content was higher in 2015 compared to 2014 at both sites. Increasing soil moisture has been reported to decrease disease severity of *R. solani* in various crops (Lootsma and Scholte 1997; Kumar et al. 1999; Gill et al. 2001) by increasing suppression of *R. solani* due to enhanced microbial activity. To conclude, the overall low disease severity and the lack of a variety effect at Gö14 and Gö15 cannot be sufficiently explained by soil temperature and moisture. Probably, soil suppressiveness, due to a microbial community structure with a higher amount of antagonistic microorganisms than in non-suppressive soils, may have been the key factor. The antagonistic potential of fungal mycoparasites like *Trichoderma* spp. (Grosch et al. 2006) and *Verticillium* spp. (Velvis et al. 1989) has been demonstrated previously.

With regard to the effect of cultivation treatment on disease severity, our data show differences between soil tillage treatments but not between silage maize and grain maize as preceding crops, although sugar beet leaves and residues of susceptible preceding crops

remaining in the field were previously reported to increase disease severity of subsequently grown sugar beet (Ruppel et al. 1985; Dircks et al. 2010). This may lead to the conclusion that the impact of soil tillage superseded the potential effect of crop residues, or that maize generally increases the disease severity of subsequently grown sugar beet independent from residue presence.

The cultivation treatment W+SM significantly decreased white sugar yield at Gö15 and Ha15, which was obviously due to the delayed achievement of the leaf area index of 3 required for maximal light interception (Andrieu et al. 1997; Malnou et al. 2006) in W+SM (September) compared to other treatments (June (Ha15) and July (Gö15)). The soil in W+SM treatment was characterized by a higher soil strength as indicated by higher penetration resistance and air capacity compared to the non-wheeled treatments. Similar effects of soil compaction on canopy formation and sugar beet yield have previously been reported by Jaggard (1977), Brereton et al. (1986) and Koch et al. (2008). However, in our study the yield decline with increasing penetration resistance was much more pronounced compared with the studies of Koch et al. (2008, 2009), which were conducted under similar soil and climatic conditions but without *R. solani* infestation. Thus, we assume that in our study the steeper decrease in yield was caused by an additional effect of the *R. solani* infection.

At Gö15 and Ha15, the slopes of the regression between disease severity and white sugar yield did not differ between the sugar beet varieties within each of the two environments, indicating that the disease-loss relation (decrease in white sugar yield per increment of increase in disease severity) was similar in both varieties, which was also observed by Buddemeyer and Märländer (2005). Nevertheless, an advantage of growing a resistant variety clearly results from the limitation of the disease severity to a lower level, which was 0-15% for the resistant variety compared to 20-50% for the susceptible variety at Ha15. Consequently, the soil inoculum potential of *R. solani* (Schulze et al. 2016) is likely to

increase to a lower degree when growing a resistant variety, which reduces the disease risk in subsequently grown host crops.

Increasing penetration resistance clearly increased the disease severity at Gö15 and Ha15 which was likely due to denser colonies of *R. solani* produced in compacted compared to well-structured soil as reported by Otten et al. (1999) and Harris et al. (2003). Thereby, the probability of an infection and the severity of the fungal attack to adjacent plants increases (Gilligan and Bailey 1997). In contrast, non-compacted soil with high volume of well-connected large pores allows for a more rapid and wider spatial spread of *R. solani* within fields (Otten et al. 1999). Furthermore, at Ha15 disease severity increased more sharply with increasing penetration resistance in the susceptible than in the resistant variety, indicating that the plant's ability to withstand fungal attack and proliferation in the taproot tissue was likely lower for the susceptible compared to the resistant variety. At a low disease severity level (both varieties at Gö15 and resistant variety at Ha 15), however, the increase in disease severity with increasing penetration resistance was similar for both varieties. Overall, our results show for the first time that penetration resistance as a specific physical parameter has a negative impact on *R. solani* disease severity of sugar beet grown in the field. It can be hypothesized that with a further increase of penetration resistance the linear relationship between penetration resistance and disease severity would at least be maintained or probably become exponential.

5. Conclusion

In our study, penetration resistance was identified to be the soil physical parameter with major impact on both yield and Rhizoctonia crown and root rot disease severity in sugar beet. We suggest that increasing penetration resistance led to denser *R. solani* colonies with a higher probability to infect the plant due to a more frequent spatial coincidence of the plant and the pathogen. However, the large variation of the data implies that environmental factors other

than penetration resistance play also a major role for Rhizoctonia crown and root rot occurrence. Our results emphasize that assuring a good soil structure and avoiding soil compaction by adequate tillage practices is crucial for R. *solani* control in sugar beet. Moreover, the cultivation of a resistant variety is important to achieve a good yield under conditions with high disease risk. Further research is required to elucidate the relationship between soil structural properties and Rhizoctonia crown and root rot occurrence at higher disease severity levels and a broader range of soil and climatic conditions.

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5. Epilog

Integrated control of Rhizoctonia crown and root rot in sugar beet

5.1 Significance of agronomic measures to control Rhizoctonia crown and root rot in sugar beet

Integrated disease control strategies generally aim at reducing the application of chemical pesticides to a minimum necessary to maintain pest populations or diseases below their respective thresholds where economically important damages or yield losses are expected. Therefore, biological, biotechnological, plant-breeding and agronomic measures are used for disease prevention issues (BMEL, 2013).

At the beginning of this century, Büttner et al. (2002) developed a concept for an integrated control strategy against the Rhizoctonia crown and root rot of sugar beet. This approach was foremost based on the cultivation of a resistant sugar beet variety supported by fungicidal protection of the seeds used and agronomic measures, such as soil tillage or a crop rotation design with a small proportion of *R. solani* host crops, e.g. maize (Fig. 1).

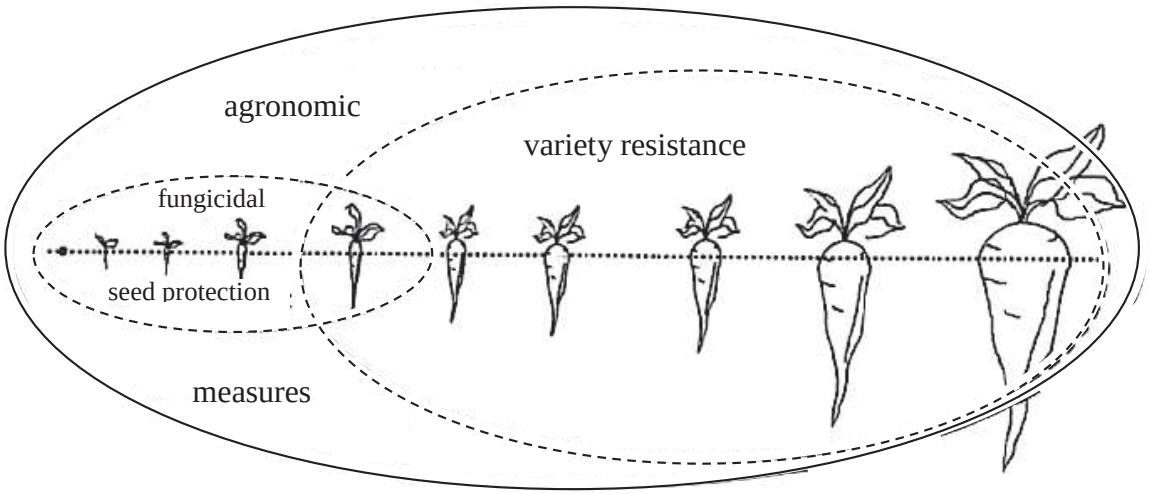

Fig. 1 Concept of an integrated control strategy against the Rhizoctonia crown and root rot of sugar beet (source: modified from Büttner et al. 2002).

Implementation of this concept in agricultural practice was, unfortunately, only partially successful: Although the first resistant sugar beet varieties were registered in 2001 (Büttner et al. 2002; Märländer et al. 2003), the development and registration of a seed treatment against Rhizoctonia crown and root rot is still lacking in Europe. Studies conducted in the USA reported that seed treatments are effective to control early ‘damping-off’ only, but

showed no positive effect on crown and root rot caused by *R. solani* due to its short duration of action (Windels and Brantner 1997; 2002; 2003). Thus it can be concluded, that a combination of a fungicidal seed treatment and a resistant variety as proposed by Büttner et al. (2002) is not feasible to control the disease in European sugar beet production areas in the near future. However, in contrast to the low efficiency of seed treatment, chemical control by a fungicide application to the soil surface at BBCH14 (Jacobsen et al. 2004) was reported to be very effective to reduce disease severity of Rhizoctonia crown and root rot in the USA (Kiewnick et al. 2001; Stump et al. 2002, 2004). First results from field experiments conducted in Germany confirmed the positive effect of fungicides for disease control (Bartholomäus et al. 2016a, 2016b). Nevertheless, due to the fact that the mode of action and the effect of the active ingredients of the fungicides to the environment has not been completely elucidated, there are no registered fungicides with an indication for Rhizoctonia crown and root rot in sugar beet in Germany to date. Consequently, in addition to variety choice, agronomic measures will likely maintain their high importance in preventing sugar beet crops from Rhizoctonia crown and root rot infection in the future.

Following a presentation given by Büttner et al. (2002), Figure 2 summarizes factors affecting the occurrence and the severity of Rhizoctonia crown and root rot of sugar beet. Generally, measures weakening the host simultaneously favor the pathogen (Büttner et al. 2002). Therefore, *R. solani* is often described as a secondary (weakening) plant-pathogen (Hoffmann et al. 1994; Wharton et al. 2007; Schulte and Horváth 2012) and a severe disease severity in sugar beet is mainly observed when the growing conditions for the crop are not favorable, e.g. at compacted soils (traffic lanes, headlands), when sowing was delayed or heavy rainfall occurred.

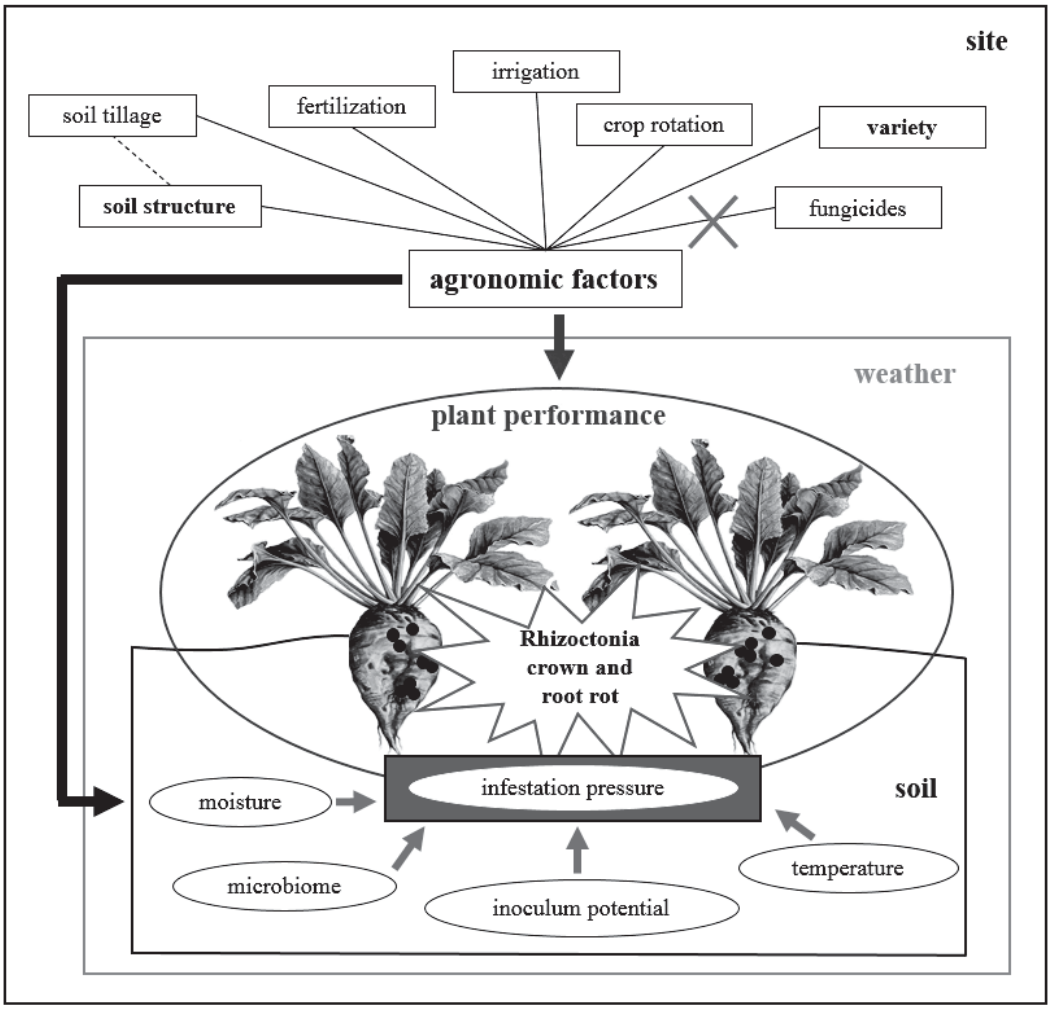

Fig. 2 Agronomic factors influencing disease occurrence and severity of the Rhizoctonia crown and root rot of sugar beet within an environment. Red X indicate that fungicides are not available for farmers because they are not registered with an indication for Rhizoctonia crown and root rot in sugar beet in Germany.

Tab. 1 Analysis of variance for the effects of environment (site × year), cultivation method (tillage (plow, cultivator, wheel traffic) × preceding crop) and sugar beet variety on the amount of *R. solani* DNA in the soil after sugar beet harvest (October), 4 environments, 2014-2015. Soils were artificially inoculated with *R. solani* (barley inoculum).

	Rhizoctonia solani DNA amount in the soil after sugar beet harvest	
	F-value	*P*-value
Environment (E)	11.376	< 0.0001
Variety (V)	17.102	< 0.0001
Cultivation method (CM)	0.799	0.5483
E x V	4.866	0.0033
E x CM	1.571	0.1121
V x CM	0.539	0.7074
E x V x CM	2.307	0.0121

The results of the present study confirmed that the environment is the most important factor affecting the disease severity of sugar beet (Schulze et al. 2016c) as well as the *R. solani* inoculum in the soil when the preceding crop maize was artificially inoculated (Tab. 1). Within the environment, the weather conditions substantially influence plant performance, soil microclimate and pathogen dynamics (Fig. 2).

It was shown that higher soil temperature positively affects Rhizoctonia crown and root rot disease severity (Bolton et al. 2010). For the soil moisture content there is no consensus in literature: Disease development is favored by a high moisture due to a higher root colonization of *R. solani* under moist conditions (Shehata et al. 1984; Lootsma and Scholte 1997; Gill et al. 2001a, 2001b). In contrast, growth and spread of *R. solani* is favored by drought leading to an increasing disease incidence and severity in dry soils (Smiley et al. 1996). This may probably enhance the possibility of a *R. solani* infection resulting in an increase of the disease severity (Gilligan and Bailey 1997). In contrast, we found that soil temperature and soil moisture did not affect the disease severity in sugar beet when the preceding crop maize was artificially inoculated (Schulze et al. 2016c) so that it remained unclear which specific combination of weather conditions may lead to increasing disease risks in the field. At this point it can be hypothesized that the weather conditions are less significant for an infection, or that its influences were superimposed by effects of e.g. the soil microbiome. The composition of the soil microbiome and changes in its composition affect the conduciveness of a soil to *R. solani*-caused diseases by an increasing antagonistic potential of the soil (Velvis et al. 1989; Grosch et al. 2006; Anees et al. 2010). However, there is no practicable way to make use of antagonists for disease control in agricultural practice.

Furthermore, agronomic factors that can be completely controlled by the farmer have an impact on both plant performance and soil (structural) conditions and play, as previously said, a decisive role in disease control: As already shown by Buddemeyer and Märländer (2005) and Buhre et al. (2009) we confirmed in the present study that the choice of the sugar beet

variety is one of the most important and effective agronomic measures to control Rhizoctonia crown and root rot in the field as the disease severity was lower when a resistant sugar beet variety was cultivated compared to a susceptible variety. However, the lower yield performance of about 10 % of a resistant in comparison with a susceptible variety under non-diseased conditions (Büttner et al. 2002) was not compensated by plant-breeding efforts until now. This led to the common decision of farmers to grow resistant varieties only in high disease risk areas, such as Lower Bavaria, where a higher yield loss of a susceptible compared with a resistant variety is expected. Thus, in such regions growing a resistant variety has an economical advantage, while in areas only occasionally affected by *R. solani*-susceptible varieties give better economic returns in most years, but may result in high losses in some years with high disease occurrence.

Besides susceptibility of the sugar beet variety grown, soil compaction was shown to negatively affect sugar beet growth and yield (Jaggard 1977; Brereton et al. 1986; Schulze et al. 2016c) and positively affect Rhizoctonia crown and root rot disease severity (Buddemeyer and Märländer 2004; Schulze et al. 2016c). Nevertheless, there was no study unraveling correlations between individual soil physical parameters and disease severity of Rhizoctonia crown and root rot in sugar beet to date. A study by Kühn et al. (2009) showed that soil structural parameters (e.g. bulk density, soil texture) did not differ between diseased and non-diseased areas but that the soil C/N ratio was significantly lower in diseased areas.

The results of the present study extend the knowledge on the impact of soil structural properties on Rhizoctonia crown and root rot disease severity: It was clearly shown that the soil structure, specifically an increasing penetration resistance, significantly increased the disease severity of sugar beet and decreased the plant performance when the preceding crop maize was artificially inoculated with *R. solani* (Schulze et al. 2016c). Therefore, the soil structure was included in Figure 2 as a particular component of the agronomic factors. This outcome strongly confirms that *R. solani* is a secondary (weakening) parasite with a higher

competiveness against the plant under environmental stress conditions weakening the plant's vitality; in this study due to a higher penetration resistance as a result of soil compaction.

5.2 Quantification of the *R. solani* inoculum potential in the soil

Quantification of the incidence of a pathogen in the soil is of major importance in disease prediction. However, the prediction of Rhizoctonia crown and root rot incidence and severity is challenging due to its heterogeneous distribution in soils within and among fields. For certain, the significant impact of the environment (site × year) on the *R. solani* inoculum potential in the soil (Tab. 1) and the lack of knowledge on which environmental parameters affect the inoculum potential in particular, makes it nearly impossible to predict the occurrence and severity.

Besides a disease prediction, the quantification of pathogens in soils can help to provide information about their behavior under different environmental conditions: We clearly demonstrated for the first time that cultivation of a susceptible sugar beet variety resulted in a significantly higher *R. solani* inoculum potential in the soil compared to a resistant variety (Schulze et al. 2016b). This difference was subsequently equalized by cultivation of the nonhost crop winter rye. In a subsequent study, the effect of a susceptible and resistant variety on *R. solani* DNA in soil was confirmed for environments with natural infestation of *R. solani* root and crown rot (Fig. 3). In contrast, the *R. solani* inoculum potential did not differ between the two varieties at environments without natural *R. solani* infestation.

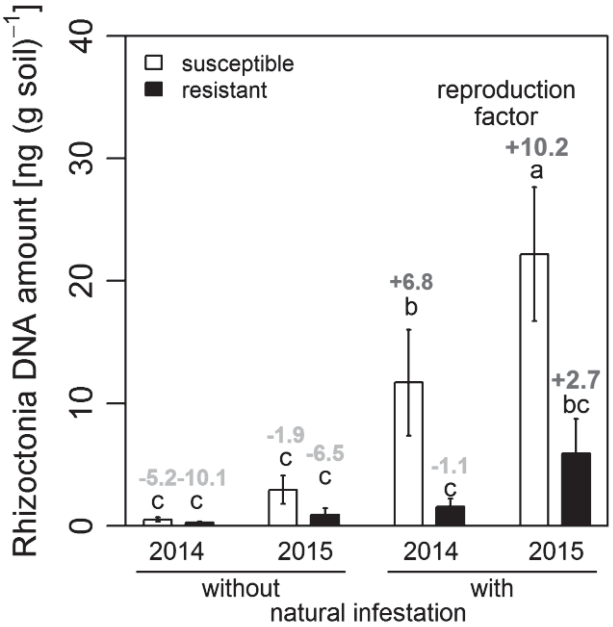

Fig. 3 Effect of a susceptible and a resistant sugar beet variety on *Rhizoctonia solani* DNA in the soil after sugar beet harvest in October without and with natural infestation with Rhizoctonia crown and root rot in 2014 and 2015 and its reproduction factor (quotient of the initial inoculum potential (sowing) and the final inoculum potential (harvest)). Plots were artificially inoculated with *R. solani* (barley inoculum in the preceding crop maize. Bars are plot means ± SE, n=20. Different letters indicate significant differences across all means displayed ($P \leq 0.05$; LSD-test).

Thereby, the significance of the interaction of environment and variety on the inoculum potential in the soil is confirmed (Tab. 1). Furthermore, the inoculum potential was increased from sowing to harvest when growing a susceptible variety at the environments with natural *R. solani* infestation and artificial inoculation of the preceding crop maize, indicated by a positive reproduction factor (Fig. 3). However, at environments without natural *R. solani* infestation and artificial inoculation of the preceding crop, the inoculum potential was decreased, indicated by a negative reproduction factor. When growing a resistant sugar beet variety, the inoculum potential remained constant or was decreased in both environments, with exception of the resistant variety in 2015.

The results of the present study are supported by Bartholomäus et al. (2016b) who also recently showed an increasing *R. solani* inoculum potential when a susceptible variety was grown. To conclude, differences in the inoculum potential between environments indicates that disease risk in general depends on environmental conditions, which is in accordance with

common observations that the disease only occurs in selected regions (see prolog, section 1.2) but not throughout all German sugar beet production areas.

Due to the overall low mean disease severity determined in the present study (Schulze et al. 2016b; 2016c), there was no significant correlation between disease severity and *R. solani* inoculum potential in the soil at sowing. In contrast, Bartholomäus et al. (2016b) determined a disease severity up to 80 % on fields with natural *R. solani* infestation (Lower Bavaria) and revealed a much closer correlation between disease severity and *R. solani* inoculum potential since the amount of *R. solani* DNA in the soil was up to 100-times higher compared to the amount of DNA measured in this study. Obviously, the new DNA based method is suitable to quantify *R. solani* inoculum in the soil, and a disease prediction in risk areas could be possible in the future; however, a well proven threshold for the amount of *R. solani* DNA in the soil must be determined by further research projects. Additionally, Bartholomäus et al. (2016b) proposed that only a combination of a resistant variety and the application of fungicides is likely the best prevention against an accumulation of *R. solani* inoculum under conducive conditions in infested fields.

Soil tillage alters the structural properties of a soil and therefore it can have considerable impact on fungal growth and spread within a field. Nevertheless, soil tillage showed no significant impact on the *R. solani* inoculum potential in the soil in the present study (Tab. 1), despite the disease severity was affected by the soil structure. The reasons for that must be investigated in further studies. However, Pumphrey et al. (1987) reported that disease severity of bare patch of wheat was reduced by intensive tillage (e.g. plowing) due to the destruction of the mycelial network and burying crop residues. Further research is needed to elucidate tillage or long-term crop rotational effects on the inoculum potential and disease development to improve disease control.

5.3 Consequences and possibilities for the agricultural practice

The present study demonstrated some novel points for a better understanding of the occurrence of the Rhizoctonia crown and root rot to improve the agronomic control measures that can be performed by farmers: At first, the significant effect of the soil structure on disease severity of Rhizoctonia crown and root rot in sugar beet; secondly, the positive effect of growing resistant sugar beet varieties to reduce the risk for *R. solani* -caused diseases in general.

The tremendous impact of the environment on both plant growth and disease occurrence and severity cannot be directly controlled by the farmers which is why a general risk for an infestation can never be excluded. But, soil tillage should be of a high mixing and loosening intensity (e.g. plow or cultivator tillage) to avoid soil compaction. This ensures optimal growing conditions achieving vital plants that are less infected by *R. solani*. Moreover, as long as fungicides not have been registered with an indication for Rhizoctonia crown and root rot in sugar beet, growing a resistant variety is the most suitable way to decrease disease severity in sugar beet and disease risk for subsequently grown host crops because the *R. solani* inoculum potential in the soil will be kept at a low level.

6. Summary

The soil-borne and plant-pathogenic fungus *Rhizoctonia solani* J. G. Kühn is a species complex of 13 different anastomosis groups (AG) and further subgroups. Isolates of the fungus are distributed in almost all arable soils world-wide. For sugar beet, *R. solani* is the causal agent of the Rhizoctonia crown and root rot. Subgroup IIIB of AG2-2 was identified to be the most aggressive and frequently occurring subgroup. Like other soil-borne pathogens, *R. solani* is heterogeneously distributed in the soil and therefore disease symptoms generally occur in patches.

The most important factors affecting Rhizoctonia crown and root rot disease severity in sugar beet are the crop rotation, the sugar beet variety cultivated, the soil tillage performed as well as weather conditions such as soil temperature and moisture and the soil structure. However, it is not clear to which extend the soil structure, more specifically individual soil physical properties, affect the *R. solani* inoculum potential in the soil and the disease severity in sugar beet. Therefore, this study aimed to identify individual soil physical properties with a significant impact on disease severity in sugar beet. It is hypothesized that an unfavorable soil structure increases the pathogenicity of *R. solani* against sugar beet leading to a higher disease severity. Moreover, a new method to quantify the *R. solani* inoculum potential in the soil was developed and applied to study the inoculum potential during the vegetation period when a susceptible and a resistant sugar beet as well as winter rye as a nonhost plant was grown.

Soil structural properties were differentiated (i) by growing maize as a *R. solani*-susceptible preceding crop before sugar beet to vary the amount of maize residues (silage maize, grain maize) and (ii) by varying soil tillage (plowing, mixing tillage with a cultivator, shallow cultivation after soil compaction by wheeling) in two-factorial field experiments in two German sugar beet production areas (Lower Saxony without natural *R. solani* infestation and Lower Bavaria with natural *R. solani* infestation) in 2013-14 and 2014-15. Fields were

artificially inoculated with *R. solani* (barley inoculum) before maize was grown. Disease severity and yield reaction in sugar beet were assessed for a susceptible and a resistant sugar beet variety.

The present study demonstrated, that the environment (site × year) was the most important factor affecting Rhizoctonia crown and root rot disease severity and white sugar yield of sugar beet. Overall, mean disease severity was low at all environments and ranged between 5 – 28 %. Only at two environments disease severity was high enough to differentiate between the two sugar beet varieties with a significantly higher disease severity of the susceptible compared to the resistant variety. This indicated the significant impact of both the environment and the sugar beet variety on disease severity. Furthermore, disease severity was significantly higher after wheeling compared to the plow and cultivator treatment. For the first time, penetration resistance as a soil physical parameter, was identified to have major impact on Rhizoctonia crown and root rot disease severity. Both sugar beet varieties showed the same reaction in disease severity and white sugar yield to increasing penetration resistance at low-disease levels. However, when the mean disease severity was above 15 % the sugar beet´s ability to withstand a *R. solani* attack was more decreased for the susceptible compared to the resistant sugar beet variety. The pathogenicity of *R. solani* against sugar beet was probably enhanced by unfavorable soil structural conditions (increasing penetration resistance) resulting in a higher increase of the disease severity, especially when cultivating susceptible sugar beet variety. We assume that the increasing penetration resistance led to denser *R. solani* colonies in the soil. This results in a higher probability to infect the sugar beet plants. Therefore, soil compaction and tillage practices with reduced loosening and mixing intensity should be avoided in agricultural practice to reduce the risk of the Rhizoctonia crown and root rot.

To determine the inoculum potential of *R. solani* AG2-2IIIB in soils a new reliable real-time PCR based method was developed. The new method allows AG2-2IIIB-specific quantification of *R. solani* from large sample volumes of 250 g of soil. It was shown that the assay is suitable to detect *R. solani* DNA in naturally infested field soils and therefore provides an alternative to other diagnostic methods to detect soil-borne pathogens. The method was applied to study the effect of a susceptible and a resistant sugar beet variety and subsequently grown winter rye as a nonhost crop on the *R. solani* inoculum potential in field soils. Moreover, during the vegetation period, a six-fold increase of the *R. solani* inoculum potential in the soil was observed when the susceptible sugar beet variety was grown. In contrast, when a resistant sugar beet variety was grown, the inoculum potential remained constant. Growing winter rye significantly reduces the inoculum potential in the soil to the initial level. It can be concluded that the increase of the *R. solani* inoculum potential in the soil by growing a susceptible sugar beet variety might increase the risk of an elevated disease level in subsequently grown host crops and, however, a reduced inoculum potential due to the growth of a resistant sugar beet variety or a non-host crop might decrease disease risk in subsequently grown host crops. However, there was no correlation found between the soil inoculum potential and disease severity emphasizing a significant impact of environmental factors on disease severity and occurrence.

7. References

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Publications and presentations

Publications

Schulze, S., Märländer, B., Koch, H.-J. Relationships between disease severity of *Rhizoctonia solani* in sugar beet (*Beta vulgaris* L.) and different soil structural conditions caused by tillage. Submitted to *Applied Soil Ecology* (Under Review).

Schulze, S., Koch, H.-J., Märländer, B., Varrelmann, M. Effect of Sugar Beet Variety and Nonhost Plant on *Rhizoctonia solani* AG2-2IIIB Soil Inoculum Potential Measured in Soil DNA Extracts.

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Schulze, S., Koch, H.-J., Märländer, B. Einfluss der Bodenstruktur auf den Befall mit *Rhizoctonia solan*i an Zuckerrüben (*Beta vulgaris* ssp. *vulgaris*) - erste Ergebnisse.

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Schulze, S., Koch, H.-J. Der Fäule vorbeugen - Welchen Beitrag leistet eine gute Bodenstruktur?

Published in Die Zuckerrübenzeitung, Nr. 6, Dezember 2014.

A selection of talks and poster presentations

Schulze, S. Impact of different soil structures on the Rhizoctonia infestation of sugar beet. 01.-02.10.2014, Doctoral Researchers Conference of GRK 1798 "Signaling at the Plant-Soil Interface", Bad Bevensen.

Schulze, S. Einfluss der Bodenstruktur auf den Befall mit *Rhizoctonia solan*i an Zuckerrüben (*Beta vulgaris* ssp. *vulgaris*) - erste Ergebnisse. 12. Göttinger Zuckerrübentagung, 04.09.2015, Göttingen.

Schulze, S. Einfluss der Bodenstruktur auf den Rhizoctonia-Befall von Zuckerrüben (*Beta vulgaris* L.) und Quantifizierung des Rhizoctonia-Inokulumpotential im Boden mittels real-time PCR. 22.-24.09.2015, Jahrestagung der Gesellschaft für Pflanzenbauwissenschaften, Braunschweig.

Schulze, S., Varrelmann, M., Koch, H.-J. Soil structure effects on the *Rhizoctonia* inoculum potential in the soil and the *Rhizoctonia* infestation of sugar beet - Experimental concept. 20.-21.03.2014, DPG Arbeitskreis Mykologie, Aachen.

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Schulze, S., Koch, H.-J. Einfluss der Bodenstruktur auf das *Rhizoctonia*-Inokulumpotential im Boden und den *Rhizoctonia*-Befall von Zuckerrüben. 16.-18.09.2014. Posterbeitrag, Jahrestagung der Gesellschaft für Pflanzenbauwissenschaften, Wien.

Schulze, S., Koch, H.-J. Soil structure effects on *Rhizoctonia* infestation of sugar beet - concept and first results. 23.-26.09.2014, Jahrestagung der Deutschen Phytomedizinischen Gesellschaft, Freiburg.

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Curriculum vitae

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❖ Academic education

Since 10/2013	Ph.D. Program for Agricultural Science (PAG) Georg-August-University Göttingen Joint research project: „Integrierte Kontrollstrategien gegen die Späte Rübenfäule der Zuckerrübe" Thesis: "*Rhizoctonia solani* in sugar beet - Relations between soil physical properties and disease severity as well as quantification of the Rhizoctonia inoculum potential in soils"
10/2011 – 09/2013	Master Program Horticultural Science Gottfried Wilhelm Leibniz University Hannover M.Sc.-Thesis: "Influence of silicic acid on formation of exodermal casparian bands – Identification and quantification of suberin and transcriptome analysis in roots"
10/2008 – 09/2011	Bachelor Program Horticultural Science Gottfried Wilhelm Leibniz University Hannover B.Sc.-Thesis: "Influence of silicic acid on suberization of the exodermis of Si-accumulators and non-accumulators"

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07/2007	Herzog-Ernst-Gymnasium Uelzen General qualification for university entrance (Abitur)

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