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.

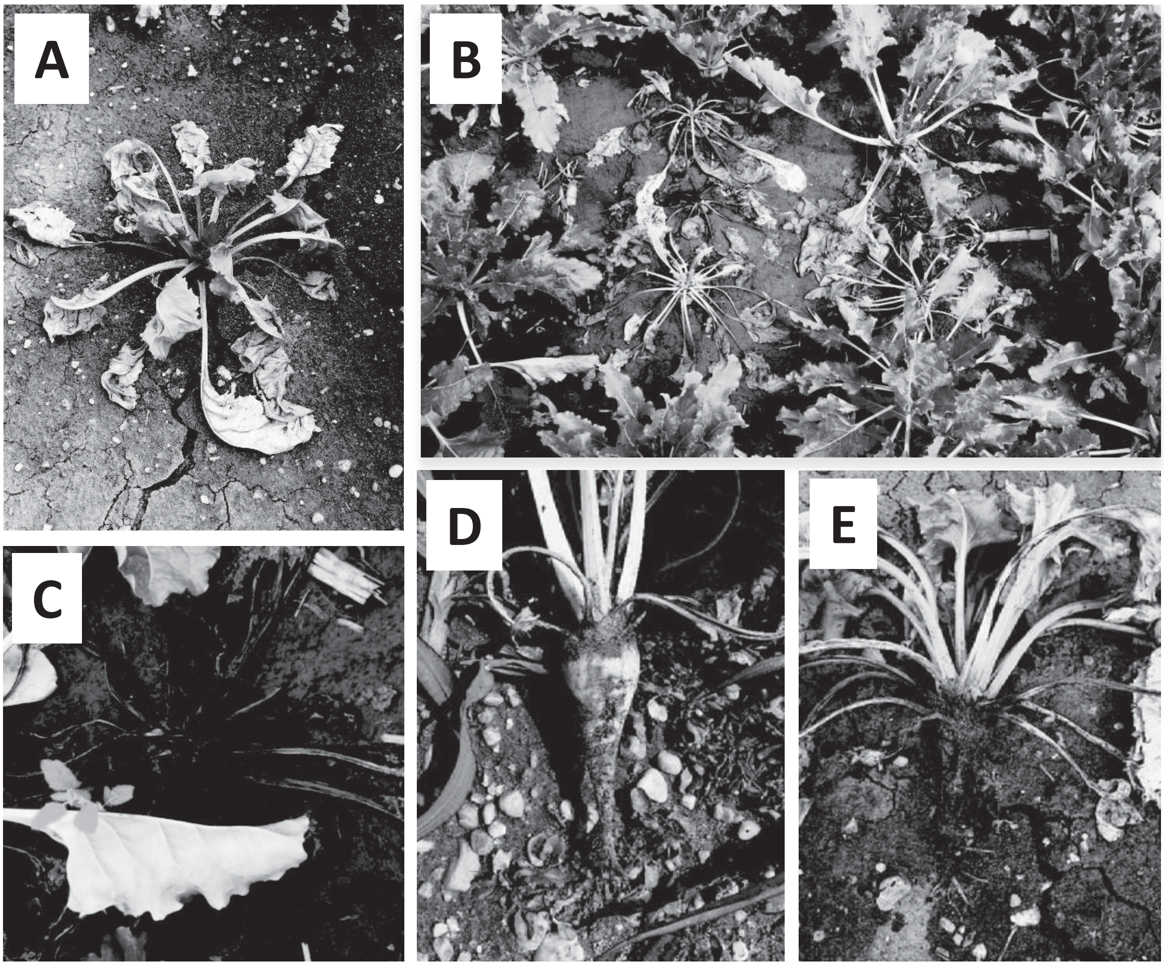

Fig. 1 Symptoms of Rhizoctonia crown and root rot on sugar beet in the field. A: First aboveground symptoms – stunted leaf growth and a dull leaf color, wilting. B: Typical disease patch with infested plants standing in close proximity to healthy plants (August). C: Black rosette of rotten leaves. D: 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-