1. Introduction

1.1 *Rhizoctonia solani*

The basidiomycete *Rhizoctonia solani* J. G. Kühn [teleomorph = *Thanatephorus cucumeris* Frank (Donk)] is an anamorph species complex and causes disease in more than 200 different plant species all over the world (Anderson 1982). In many economically important crops, like rice, soy bean, potato, corn and sugar beet, *R. solani* is one of the most devastating pathogens and induces mainly rots on the roots and occasionally also infects stems and leafs of its hosts above the ground (Cubeta and Vilgalys 1997; García et al. 2006). The pathogen invades the plant by forming infection structures and additionally secretes enzymes, which result in the degradation of the tissue (Keijer 1996, Weinhold und Sinclair 1996, Ruppel 1973). It is a soil-borne pathogen, which is able to grow saprophytically on plant debris or crop residues and can survive unfortunate conditions for long periods by forming sclerotia. Based on vegetative incompatibility, it is divided into 14 different anastomosis groups (AGs), which are further subdivided into intraspecific groups according to host range or biochemical/molecular characteristics (Cubeta and Vilgalys 1997; Carling et al. 2002, Sharon et al. 2006). Even though different anastomosis groups specifically infect certain host crops, the host range can also be very wide und does usually overlap (Arakawa and Inagaki 2014). These characteristics make *R. solani* a plant pathogen, which is difficult to control by agronomic measures (Anderson 1982; Ohkura et al. 2009).

1.2 *Rhiozoctonia solani* in sugar beet

R. solani is considered the most important pathogens in some sugar beet growing areas (Kiewnick et al. 2001, Ruppel 1972). Depending on the time-point of infection, disease caused by *R. solani* is divided into seedling damping-off in the early developmental stages of the sugar beet plants and Rhizoctonia root and crown rot, which occurs later in the season.

Younger sugar beet plants are susceptible to a variety of different AGs (O' Sullivan and Kavanagh 1991; Bolton et al. 2010). In contrast, Rhizoctonia root and crown rot is mainly caused by the AG 2-2 IIIB, but also AG 2-2 IV and AG 4 are able to induce this disease in older sugar beet plants (Bolton et al. 2010). Still, it is reported that the overall disease severity caused by AG 2-2 IV and AG 4 is lower and that induced lesions remain superficial (Rush et al. 1994; Engelkes and Windels 1996; Hanson und McGrath, 2011). Nevertheless, the fungus infects sugar beets

throughout the growing season (Kirk et al., 2008) and infection of younger sugar beet plants by each of these three AGs can results in severe damping-off (Windels and Brantner 2005).
In Europe, the AG 2-2 IIIB is the most devastating one, infecting more than 36,000 ha of the sugar beet cultivated area (Garcia et al. 2001). In contrast, the AG 4 is the prevalent species in some areas of the United States (Strausbaugh et al. 2011). However, also in the US, the AG 2-2 IIIB is responsible for the majority of economic loss due to *R. solani* infestation in sugar beet (Strausbaugh et al. 2011). For instance, in the years 1998 and 1999, an assessment of 1.4 million hectares cultivated with sugar beet, showed that *R. solani* was considered as very important in 27.2 % and 23.7 % of the area, respectively (Kiewnick 2001). Rhizoctonia root and crown rot can cause a plant mortality of up to 60 % (Allen et al. 1985) and in addition to the yield reduction, the quality of the beets as well as their storability are severely impaired (Martin 2003; Strausbaugh and Gillen 2009). In addition, beets which are affected by Rhizoctonia root and crown rot show a dramatic increase in invert sugar content, resulting in severe problems during processing in sugar refineries (Bruhns et al. 2004).

In general, warm and wet conditions promote the disease development, whereas cold temperature beneath 10 °C prevents the expression of disease symptoms (Bolton et al., 2010). The growth temperature optimum between isolates can differ significantly and is considered to range between 20-30 °C (Engelkes and Windels 1994, Rush et al. 1994). When environmental conditions are conducive, the fungus infects the beet by forming small dark lesions, which are clearly separated from the healthy tissue.
Infection can start either at the taproot of the beet, mostly near the soil surface, or in the crown. The lesions disseminate, and in most cases, a secondary infection with other pathogens occurs (Strausbaugh and Gillen 2008). The leaves show some degree of yellowing and then suddenly wilt, which results in the typical symptoms (Fig. 1 left) (Engelkes and Windels 1996). At the end, characteristic patches of completely mummified beets can be found in the fields (Fig. 1 right).

Fig. 1: Typical Symptoms of Rhizoctonia root and crown rot after inoculation. Left: Mummified beet due to *R. solani* infection; Right: Typical patch of Rhizoctonia root and crown rot.

1.3 Integrated control of *Rhizoctonia solani* in sugar beet

1.3.1 Disease management by agronomic measures

In fields with a continuous cultivation of sugar beet, *R. solani* isolates from the AG 2-2 IIIB become the dominant group and induce severe losses in the crop (Ogoshi 1987). To prevent this excessive accumulation of *R. solani* inoculum in the soil, a crop rotation with non-hosts for a minimum of three years is recommended (Schuster and Harris 1960; Ruppel 1985), since the inclusion of susceptible alternative hosts further increases disease severity in following sugar beet (Buhre et al. 2009). However, in severely infested areas, crop rotations of seven to eight years are thought to be necessary to reduce losses in sugar beet (Harveson and Rush 2002).

The host range of *R. solani* AG 2-2 IIIB is very wide and includes, apart from sugar beet, corn, soybean, bean crops, rice, mat rush, turfgrass and potato (Engelkes and Windels 1996; García et al. 2006; Muyolo et al. 1993; Summer and Bell 1982; Woodhall et al. 2015). This characteristic makes this pathogen difficult to manage solely by crop rotation. At the seedling stage its host

range is even wider including also barley, muskmelon, sorghum and wheat (Ruppel 1985). Still, those plants lose their susceptibility towards the fungus with increasing plant age, limiting their effect in crop rotations (Ruppel 1985).

In contrast, isolates of the AG 4 can induce root rot in mature wheat, which makes this crop an unsuitable pre-crop for sugar beet in those areas (Rush et al. 1994). Nevertheless, the disease severity can be significantly reduced by a careful crop rotation management (Schuster and Harris 1960; Ruppel 1985). Moreover, other agronomic measures like plowing or the cultivation of intercrops, e.g. mustard, can decrease the *R. solani* infestation in sugar beet (Buhre et al. 2009). In fields, which are artificially irrigated the disease severity of Rhizoctonia root and crown rot can be reduced further by careful management of the supplied water, since higher soil moisture levels promote disease development (Harveson and Rush 2002). Also early planting of sugar beets, when the temperatures are lower, is considered as suitable measure to reduce losses due to *R. solani* induced by seedling damping-off. Furthermore, as resistance development is correlated with plant age, early planting of resistant cultivars as well could positively reduce the disease severity of later infections (Engelkes and Windels 1994).

In general, cultivation of resistant cultivars is the key factor using agronomic measures for control of *R. solani* in sugar beet. Resistance breeding against *R. solani* started already in the early 1960s (Panella 1998). Nowadays many of the available cultivars, which show resistance characteristics towards *R. solani*, are based on the two resistant breeding lines developed in this initial breeding program (Panella and Ruppel 1996). First analysis of the *R. solani* resistance indicated that it is a quantitative resistance, which is affected by epistatic interactions and involves two gene loci and two to three alleles (Hecker and Ruppel 1975). It is proposed that a dosage effect applies, since 3x-hybrids displayed a higher level of resistance than 2x-hybrids (Hecker and Ruppel 1976). Detailed analysis of the resistance revealed three quantitative trait loci (QTL), which were responsible for 71 % of the observed variation in resistance of resistant cultivars (Lein et al. 2008). The QTLs were identified to be located on the chromosomes 4, 5 and 7 (Lein et al. 2008).

However, the knowledge about the molecular mechanisms behind this resistance is limited. Taheri and Tarighi (2011) showed that the application of riboflavin induced resistance in sugar beet plants against *R. solani*, which in turn was correlated with an increase of the H_2O_2

concentration in the plant tissue. Using the model organisms *Arabidopsis thaliana,* Foley and co-workers (2013) supported this finding by demonstrating that the resistance towards AG 8 depends on the formation of reactive oxygen species. Furthermore, Taheri and Tarighi found that also the accumulation of phenolic compounds is linked to plant resistance towards *R. solani* in sugar beet. Based on their findings they conclude that the stimulation of the phenylpropanoid pathway, connected with an increase in the phenylalanine ammonia lyase (PAL) activity, plays an important role in the protection against *R. solani* (Taheri and Tarighi 2011). This is also supported by findings of Beta and Purkayastha (1999), who showed that the inhibition of PAL increased the susceptibility of rice towards *R. solani*. However, those findings are probably only a part of the puzzle, since studies on the metabolome of susceptible and resistant cultivars in regard to *R. solani* infection suggest a complex role of primary and secondary metabolites including alkaloids, terpenes and phytoalexins, in pathogen defense (Webb et al. 2016).

Due to the complex breeding system of sugar beet, the breeding of disease resistant germplasm takes between 8 and 15 years, which complicates the process to make improved resistant cultivars available to the market (Panella and Lewellen 2007). However, a screening of almost 700 Beta accessions showed that between 5 and 7 % possess resistance traits towards Rhizoctonia root and crown rot. This includes highly resistant accessions of garden beets and unspecified *B. vulgaris spp*., which could be used as source for novel resistance genes (Luterbacher et al. 2005). Anyhow, the resistance of currently available cultivars does not completely prevent the infection with Rhizoctonia root and crown rot (Panella et al. 2008; Büttner et al. 2004). Furthermore, it is connected with a yield penalty under non-disease conditions (Büttner 2002; Panella and Ruppel 1996), which clearly demonstrates the need for improved cultivars.

In conclusion, agronomic measures comprising crop rotation, growth of resistant cultivars and tillage practices can significantly reduce the disease severity of Rhizoctonia root and crown rot, but are not able to completely prevent losses due to the disease (Buhre et al. 2009).

1.3.2 Chemical control of *Rhizoctonia solani*

The research on chemical control of *R. solani* started in the United States already in the 1950s (Afanasiev and Morris 1952). However, it took almost 50 years (1999) until the first fungicide, containing the active ingredient azoxystrobin (AZ), was officially labeled for the control of

seedling damping-off as well as Rhizoctonia root and crown rot in sugar beet (Kiewnick et al. 2001). Since then various studies demonstrated the excellent efficacy of AZ, formulated as Amistar (Syngenta), making it the most important compound for the control of *R. solani* in sugar beet in the United States (Stump et al. 2002; Stump et al. 2004; Windels and Brantner 2005; Kirk et al. 2008; Bolton et al. 2010; Barnett et al. 2011, Noor and Kahn 2015; Liu and Khan 2016). Anyhow, reports of *R. solani* isolates, including the AG 2-2 IIIB, showing resistance towards the AZ (Olaya et al. 2012; Djébali et al. 2014; Blazier and Conway 2004), indicate that this control strategy should be reconsidered. Yet, none of the tested alternatives showed a similar control efficacy using a similar application dosage (Bolton et al. 2010; Liu and Kahn 2016). In contrast to the US, no fungicides for the control of *R. solani* in sugar beet are approved in the European Union. For a detailed review regarding the chemical control of *R. solani* in sugar beet see manuscript II.

1.3.3 Biological control Rhizoctonia *solani*

Due to the limited availability of efficient fungicides and the insufficient disease control achieved by agronomic measures alone, many studies focus on biological control of *R. solani*. Most of this research is inspired by the so-called phenomenon of suppressive soil, which is also found in sugar beet (Hyakumachi et al. 1990). In those soils, no considerable disease develops in the host plant even though the pathogen is present and environmental conditions are conducive. Many factors have been speculated to be involved in the observed suppressiveness including mycoparasitism, specific antagonisms as well as virus infections (Papavizas and Lumsden 1980). It was the aim of many studies to promote or induce the suppressiveness of soils. Postma and colleagues showed that the supplementation of compost into soil could sufficiently suppress seedling damping-off caused by *R. solani* in sugar beet (Postma et al. 2003). Also the incorporation of protein-rich amendments increased the suppressiveness of soils towards *R. solani* in sugar beet (Postma and Schilder 2015). Even though, many studies demonstrated that the ability of *R. solani* to cause disease is significantly affected by the microbial community in the soil, the management of this complex system is difficult and effects obtained are variable or often only short-termed (Anees et al. 2010; Postma et al. 2003). In some cases, attempts to induce soil suppressiveness had also negative effects because new pathogens were introduced by the supplementation with compost (Kinkel et al. 2011 and references therein; Hoitink et al. 1997 and references therein).

Identifying the most important organisms responsible for disease suppression and their targeted use is therefore a straightforward way of biological control compared to unspecific alteration by the application composts or other soil amendments. Analysis of the microbial communities associated with the rhizosphere of sugar beets showed that about 11 % of the bacteria and 14 % of fungi possessed antagonistic abilities against *R. solani* (Zachow et al. 2007). Bacteria, which are known to be antagonistic to *R. solani*, belong to different genera e.g. *Lysobacter*, *Bacillus* or *Pseudomonas* (Mendes et al. 2011; Postma et al. 2010; Kiewnick et al. 2001; Ogoshi 1987 and references therein) and some of them have been successfully used to reduce *R. solani* in sugar beet (Mendes et al. 2011; Kiewnick et al., 2001).

Efficient biocontrol in sugar beet was also induced by the supplementation of different yeast species or mycoparasitic fungi (El-Tarabily 2004; Allen et al. 1985; Ruppel et al. 1983, Abada 1994). Attempts to control *R. solani* with artificially introduced *Laetisaria arvalis*, demonstrated that this led to a significant short term reduction of the *R. solani* inoculum. Still, the concentration of the mycoparasite decreased after some month followed by a build-up of the *R. solani* inoculum back to the initial level (Allen et al. 1985). *Trichoderma harzianum* is another mycoparasite of *R. solani* and has also been reported to significantly reduce the disease severity of Rhizoctonia root and crown rot within the growing season when applied prior sowing (Ruppel et al. 1983, Abada 1994).

In addition, mycofumigation seems to be another possible way to reduce disease severity of *R. solani*. Stinson and colleagues showed that supplementation of soil with fungal isolates of *Muscodor albus* and *M. roseus*, which produce antimicrobial volatiles, significantly decrease disease incidence on sugar beet seedlings inoculated with *R. solani* (Stinson et al. 2003). Biofumigation, based on the release of toxic substances from disrupted plant tissue, which is incorporated into the soil, seems to be another possible way to reduce soil borne pathogens like *R. solani* (Matthiessen and Kirkegaard 2006). In sugar beet, the incorporation of brown mustard, grown as an intercrop, showed a significant reduction in the disease severity of Rhizoctonia root and crown rot (Motisi et al. 2009, Motisi et al. 2013). Still, this effect might simply be due to the enrichment of organic matter by the incorporation of the plant residues into the soil, leading to a promotion of the bacterial community instead of biofumigation (Kasuya et al. 2006). Results from Kasuya and co-workers (2006) indicate that residues of different plants affect the microbial

community in the soil in different ways, since some plant species promoted fungal antagonists and others led to an enrichment of the bacterial community. Nevertheless, also *R. solani* is able to live saprophytically on plant debris and certain crop residues promote the build-up of inoculum (Ruppel 1985).

Another biocontrol approach is the usage of hypovirulent species of *R. solani* (Herr 1995 and references therein). Greenhouse and in-field experiments with hypovirulent *R. solani* isolates and binucleate *Rhizoctonia spp*. demonstrated their efficacy to reduce Rhizoctonia root and crown rot in sugar beet (Herr 1988; Webb et al. 2015). Here, the ability to efficiently colonize the beet seems to be an important trade for a successful biocontrol (Herr 1988). The mechanism by which hypovirulent *R. solani* isolates hinder the infection of virulent ones is not fully elucidated, but neither mycoparasitism nor antibiosis seems to be involved (Herr 1995 and references therein). In contrast, it is hypothesized, that the disease reduction is either caused by direct competition (Cook and Baker 1983; Sneh et al. 1989) or induction of systemic resistance in the plants (Xue et al. 1998). Also the possible involvement of mycoviruses is speculated (Castanho and Butler 1978).

In conclusion, even though many promising biocontrol agents have been identified, which show good control efficacy under laboratory conditions, their efficacy under field conditions is not or only poorly studied. The complex interaction between biocontrol agents and their environment, including chemical and physical soil properties as well as the microbial soil community, make them difficult to manage (Kinkel et al. 2011) and leaves them less reliable compared to chemical protection (Ruppel et al. 1983). Still, a great potential exists and further research might allow the identification of novel biocontrol agents showing the same solid and easy handling of chemical treatments and in parallel reduce the risk for non-target organisms.

1.4 Biological control by mycoviruses

Research on mycoviruses started in the early 1950s when Sinden and Hauser reported a serious disease in cultivated mushrooms, which they named “La France Disease”. They speculated that it is caused by mycoviruses (Sinden and Hauser 1950). However, it was not until 1962 that the first successful purification of virus-like particles from a fungus (*Helminthosporium victoria*) was reported (Hollings 1962). Still it was the finding that a mycovirus, identified in fungal extracts of

Penicillium stoloniferum, was responsible for interferon induction in animals, which induced the first boost in mycovirus research (Ellis and Kleinschmidt 1967).

By 1972 a total of 55 mycoviruses from all major fungal taxa had been described (Bozarth 1972). Lemaire and colleagues were among the first researches, who tried to exploit viruses for biocontrol approaches. They found that a virus infected hypovirulent strain of *Ophiobolus graminis* (today *Gaeumannomyces graminis*) could reduce disease severity caused by virulent isolates, when both were applied in mixture. This encouraged also other scientists to search for similar phenomena (Bozarth 1972). However, the initial enthusiasm decreased when it was reported that isolates of *Helminthosporium* infected with mycoviruses were more aggressive than strains without mycoviruses, which indicated that there was no general effect of mycoviruses on their hosts (Bozarth 1972).

Today we know that most mycovirus infections do not cause visible symptoms in their fungal hosts. The tendency to a cryptic life style of mycoviruses is not surprising since they are thought to have no extra-cellular phase and in turn depend on the survival of the host (Pearson et al. 2009). However, attempts to use mycoviruses for the benefit of humans were still ongoing and promoted by the finding that certain mycovirus encode killer toxins. Those mycoviruses have been discovered in different yeast species. They allow their host to secrete proteins lethal to other isolates of the fungus not carrying the respective virus (Woods and Bevan 1968; Kandel and Koltin 1978; Philliskirk and Young 1975). Today, those viruses are widely used to combat contaminating yeast species in the preservation of food or during fermentation processes (Palpacelli et al. 1991; Hara et al. 1980).

Also unrelated plant pathogenic fungi including *R. solani*, which proved to be very sensitive, have been shown to be affected by killer toxins secreted by certain yeast isolates (Walker et al. 1995). However, no further studies were undertaken to use these toxins as alternative to chemical fungicides to combat *R. solani*. Today, most of the research regarding mycoviruses is still carried out to identify potential biocontrol agents and so far numerous mycoviruses have been reported to induce hypovirulence in a variety of different plant pathogenic fungi (e.g. Moleleki et al. 2003; Castro et al. 2003; Wu et al. 2007; Xie et al. 2006; Lee et al. 2011; Zheng et al. 2014; Zhai et al. 2016; Xie et al. 2016). Still, the difficulty in fulfilling the Koch's postulates remains and

significantly reduces the number of mycoviruses, which indubitably induce hypovirulence in their host (Pearson et al. 2009).

Nevertheless, a few successful examples of pathogens controlled by mycoviruses exist. The most prominent is the one of chestnut blight caused by the ascomycete *Cryphonectria parasitica*. When the pathogen started to spread in 1904, all efforts to control the disease, including quarantines, breeding and chemical treatments failed and the fungus almost destroyed the entire population of chestnut trees in the United States (Anagnostakis 1982). In 1938 chestnut blight was also reported in Europe, but in contrast to the United States, some trees recovered after an infection (Anagnostakis 1982). Fungal isolates derived from recovered trees displayed a changed phenotype including irregular growth and hypovirulence (Anagnostakis 1982). Grente and colleagues found these alterations to be cytoplasmically determined and readily transmitted between related isolates (Anagnostakis 1982). In 1973 first attempts to use the hypovirulent strains from Europe to control the fungus in the US were made and demonstrated the ability of the approach to cure infected trees. However, control efficacy was limited by vegetative incompatibility, which prevented the spread of the mycoviruses into natural populations of the pathogen (Anagnostakis 1982). Due to the lack of virus like particles, it took until 1977 when Day and co-workers (1977) established that viral dsRNA was associated with the observed hypovirulence phenotype. Today we know that this hypovirulence is induced by different hypoviruses which became the best-studied example in mycovirus research, e.g. by the transmission via infectious cDNA-clones (Shapira et al. 1991; Choi and Nuss 1992; Nuss 2005 and references therein). However, critical voices point out the limited success of mycoviral control of chestnut blight in natural forests, since the artificially introduced hypoviruses were not able to spread in the native population of the fungus in the US (Milgroom and Cortesi 2004). The limited transmission of mycoviruses due to vegetative incompatibility of their host– which is hypothesized to be an evolutionary adaptation to combat mycoviruses (Choi et al. 2012) – is considered as one of the main obstacles using mycoviruses as biocontrol agents (Son et al. 2015; Xie and Jiang 2014).

Nevertheless, a few reports clearly show the potential to overcome this barrier, for example those were the host range of mycoviruses was expanded by the transmission into protoplasts resulting in hypovirulence in the new host species (Kanematsu et al. 2010; Lee 2011) or the successful

transmission of mycoviruses via hyphal contact between vegetative incompatible groups (Xiao et al. 2014; Xie and Jiang 2014; Liu et al. 2015). A special class of mycoviral biocontrol agents is Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1), which has been shown to be infectious as particles and is readily transmitted unaffected by vegetative incompatibility (Yu et al. 2010). Further analysis indicated that this virus represents a very promising agent for biological control, since spray application of purified particles, as well as of hyphal fragments from infected fungi, efficiently controlled rapeseed stem rot under field conditions (Yu et al. 2013).

Another approach to overcome the barrier of vegetative incompatibility was followed by Zhang and Nuss, who genetically engineered an isolate of *C. parasitica* to be a superior donor strain for mycoviruses (Zhang and Nuss 2016). Furthermore, Xie and Jiang conclude that in contrast to a natural system with its high species diversity, the homogenous environment in agricultural fields often facilitates the accumulation of a certain genetically uniform fungal pathogen. This in turn might facilitate also the spread of the viruses, which could be easily introduced by the application of compatible infected strains at the right time (Xie and Jiang 2014).

Even though no real breakthrough in the area of biocontrol via mycoviruses has been achieved yet, all these findings might help to use mycoviruses as efficient and environmental safe control measure for fungal plant pathogens one day. Furthermore – and technically as a side effect – the current attempts have revealed many interesting aspects of these viruses regarding taxonomy, ecology and evolution. For example, due to the identification and official acceptance of hypoviruses, virologist came to the conclusion that viruses do not necessarily possess a capsid, which used to be the definition of viruses (Murphy et al. 2012; Raoult and Forterre 2008). Today, research on mycoviruses, inspired from the search for biocontrol agents, has uncovered that mycoviruses are presented in all major phyla of the fungi and the majority possess an RNA genome, which is either single or double stranded (Son et al. 2015). So far, twelve different mycovirus families have been approved by the ICTV (Table 1), but several more families are proposed and the speed in which novel mycoviruses are being discovered indicates that this number will strongly increase in the future (Ghabrial et al. 2015).

Table 1: Overview of families containing mycoviruses recognized by the ICTV (ICTV 2016; Ghabrial et al. 2015).

Family	Recognized species	Genome	Capsid
Alphaflexiviridae	2	ssRNA; one segment	in some cases
Barnaviridae	1	ssRNA; one segment	yes
Chrysoviridae	8	dsRNA; four segments	yes
Endornaviridae	3	dsRNA; one segment	no
Gammaflexiviridae	1	ssRNA; one segment	yes
Hypoviridae	4	ssRNA; one segment	no
Megabirnaviridae	1	dsRNA; two segments	yes
Narnaviridae	7	ssRNA; one segment	no
Partitiviridae	25	dsRNA; two segments	yes
Quadrivirus	1	dsRNA; four segments	yes
Reoviridae	3	dsRNA; ten - twelve segments	yes
Totiviridae	21	dsRNA; one segment	yes

Furthermore, the exploration of the diversity of mycoviruses showed that some of them are more closely related to plant viruses than to other fungal viruses, like it is the case for the *Endornaviridae* and the *Partitiviridae* (Song et al. 2013; Nibert et al. 2014). Whereas other mycoviruses are more closely related to viruses which infect mammals (Liu et al. 2009). This gives valuable insights into virus evolution (Pearson et al. 2009 and references therein; Koonin et al. 2015). Furthermore, interesting case studies, like the one of Curvularia thermal tolerance virus (CThTV), which induces the ability to tolerate high temperature stress in its host fungus as well as in the plant the fungus inhabits (Márquez et al. 2007), indicate how diverse the role of mycoviruses can be and how much more there is to explore besides the phenomenon of hypovirulence.

1.5 Mycoviruses of *Rhizoctonia*

In 1972 the very first report about a virus found in *R. solani* was published. It was identified in a screening of different fungi to evaluate the prevalence of mycoviruses (Bozarth 1972). However, no further characterization of the virus was performed. A few years later, Castanho and Butler (1978) reported that *R. solani* was affected by a degenerative disease, which they called Rhizoctonia decline. The disease correlated with an irregular appearance, reduced pigmentation, decreased abilities to form sclerotia, a reduced growth rate and resulted in hypovirulence. They

demonstrated that these symptoms were associated with the occurrence of certain dsRNA elements, but could not identify virus-like particles (Castano et al. 1978). However, they showed that fungal isolates can be cured from the disease by hyphal tipping and that the diseased phenotype can be readily reintroduced by anastomosis (Castanho and Butler 1978).

In contrast, transmission of the disease to other *R. solani* isolates than the previously cured ones failed (Castanho and Butler 1978). Tests analyzing the biocontrol abilities of the hypovirulent isolate showed that in-furrow application efficiently reduced seedling damping-off in sugar beet caused by the cured highly virulent strains, but no tests were done using natural *Rhizoctonia* inoculum (Castanho and Butler 1978b). Additionally, the infected isolate was unable to survive in the soil for longer periods, which limited its use as biocontrol agent (Castanho and Butler 1978b). Nevertheless, these findings were a motivation for other scientists to study the effects of dsRNA elements on *R. solani* in more detail.

A screening of 50 field isolates of *R. solani*, including the anastomosis groups AG 1, AG 2, AG 3, AG 4 and AG 5 indicated that dsRNA elements are very common in this fungus, but that no general correlation between the presence of dsRNA and hypovirulence could be assumed (Zanzinger et al. 1984). Furthermore, Finkler and co-workers (1985) showed that the occurrence of dsRNA, which they found to be associated with the presence of viral particles in some cases, was correlated with virulence of the fungal host, since all hypovirulent isolates tested were free of dsRNA, whereas virulent isolates contained multiple fragments. Additionally they were able to transmit virulence to a hypovirulent isolates by the transmission of dsRNA via anastomosis (Finkler et al. 1988). After these observations, the research focused on describing the diversity of dsRNAs. The isolation of dsRNA from many different anastomosis groups of *R. solani* from all over the world and studies of their relatedness, showed that the diversity was very high, differed between different AGs and did not follow a geographical pattern (Bharathan and Tavantzis 1991; Kousik et al. 1994; Kim et al. 1996; Bharathan et al. 2005).

In 1994 Lakshman and Tavantzis reported the finding of hypovirulent isolates, which had spontaneously emerged from a virulent isolate of the AG 3. This alteration in virulence was correlated with a change in the dsRNA pattern (Lakshman and Tavantzis 1994). After further analysis, they found that three strains obtained from the virulent maternal strain possessed distinct combinations of dsRNA elements and exhibited different levels of reduced virulence.

This allowed a detailed study of the way the single dsRNA elements affect their fungal host. The authors found that the alteration of the virulence was caused by two dsRNA fragment. The first fragment of 6.4 kb increased the virulence of the host, whereas a second 3.6 kb fragment diminished virulence proportional to the virus titer (Jian et al. 1997).

Studies testing the hypovirulent isolate as biocontrol agent showed that its application efficiently reduced the disease severity of its maternal isolate. However, no control of *Rhizoctonia* species naturally infecting potato tubers in the field was achieved (Bandy and Tavantzis 1990). Detailed analysis of these two fragments showed the 6.4 kb fragment to be related to the plant bromoviruses (Jian et al. 1998). The smaller fragment of 3.6 kb, later named *Thanatephorus cucumeris mitovirus* (TcMV), belongs to the mitoviruses and interestingly a DNA copy of the viral sequence was present in its host genome (Lakshman et al. 1998). The induction of hypovirulence by TcMV is associated with the downregulation of the shikimate pathway (Liu et al. 2003). It was possible to transmit TcMV via hyphal phusion to other isolates of different anastomosis groups under laboratory conditions at very low frequencies and the transmission resulted in typically phenotypic alterations (Charlton and Cubeta 2007). However, the infection was not stable and the virus disappeared after repeated subculturing. No further analysis in regard of biocontrol was done (Charlton and Cubeta 2007).

Also in *R. solani* isolated from sugar beet, dsRNAs elements have been identified and some of them were reported to be associated with diminished or increased virulence. However, no detailed analysis of those viruses was carried out (Naeeni et al. 2012).

In the last years several new mycovirus species have been identified in *Rhizoctonia* and an increasing number of genomes became available including species of recognized viral families like *Rhizoctonia solani partitivirus 717* (Tavantzis and Bandy 1988; Strauss et al. 2000), Rhizoctonia solani partitivirus 2 (Zheng et al. 2014) and several mitoviruses (Das et al. 2014), but also viruses which belong to novel groups like Rhizoctonia solani dsRNA virus 1 (Zheng et al. 2013) or Rhizoctonia solani RNA virus HN008 (Zhong et al. 2015). The application of deep sequencing approaches further increases the number of identified genomes, indicating the huge diversity of mycoviruses that infect *R. solani* (Marzano et al. 2016). Research on the topic of mycoviruses in general and in mycoviruses of *R. solani* in particular suggest the existence of mycoviruses, which have good potential as future biocontrol agents against Rhizoctonia root and

crown rot. However, previous research also points out that the majority of the mycoviruses are not qualified for biocontrol and that extensive research is required to identify a suitable viral biocontrol agent. Finding approaches, which allow to efficiently screen hypovirulent isolates of *R. solani* for the presence of mycoviruses, like virome analysis via deep sequencing, followed by a detailed study of the way those viruses affect their host and how they can be efficiently transmitted, is most likely the most suitable approach to cope with this challenge.