FACULTY OF BIOSCIENCE ENGINEERING

BIOLOGICAL CONTROL OF *RHIZOCTONIA SOLANI* IN FIELD BEAN (*VICIA FABA* L.) USING CYCLIC LIPOPEPTIDE-PRODUCING BACTERIA

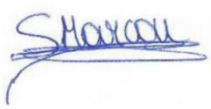
Raf Verdoodt
Student number: 01802969

Promotor: Prof. dr. ir. Monica Höfte
Tutor: ir. Shirley Marcou

Master's Dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Science in Bioscience Engineering: Agricultural Sciences
Academic year: 2022-2023

GHENT UNIVERSITY

The promotor, Monica Höfte



The tutor, Shirley Marcou

The author, Raf Verdoodt

Preface

Well, here it is then. After relentlessly working for the past couple of weeks I can finally write my preface, putting the finishing touches on my thesis and almost concluding my time as a student. For most of my life I have been going to lessons, handing in assignments, studying for tests and exams and all this concluded with the most expansive project I have ever made. These past five years have been the most challenging of my life, not only academically but also on a personal level, from the passing of my beloved grandfather to many other personal issues. While I have appreciated my time as a student, I cannot express the joy I feel knowing I can open a new chapter in my life and while I am extremely proud of what I have achieved, it would be foolish to believe I could have done it without any help.

There is really no question who helped me the most in the making of this thesis. After following a practical course from Shirley last year, I quite quickly realized she is one of the most helpful teachers I have had in my time as a student. So, when I saw an interesting subject with her as tutor, it was an obvious choice for my thesis. And she did even more than I could have ever expected. From helping me with experiments on late Friday nights or weekends, to always and quickly answering all my (mostly) redundant questions, I really could not have imagined a better tutor. Of course, prof. dr. ir. Höfte, or as I am allowed to call here, Monica, was also listed under that same subject. Following her courses, she already proved to be a great professor and educator. She further displayed these traits by providing me with useful insights and ideas to make my thesis more interesting than I could have done on my own. Both motivated and made it possible for me to finish my thesis in time, of which I am truly appreciative.

This helpfulness really seems to be a trait of the entire lab of phytopathology. Not once did I hear “no sorry, I can’t help you” or “you’ll have to figure that out on your own”. I needed but ask and everybody was always willing and able to help me out. In particular, I would like to thank Hanna, Ilse, Nadia, Ramize and Yasmine for the services they provided to aid me with certain experiments. I do not think I could have landed in a better place to carry out my thesis.

Of course, I could not have made it here without the help of my family. I wholeheartedly believe my brother has been the most instrumental in aiding me with my studies. When I was worked up or doubting myself, I could always count on him to calm me down or to reassure me of my capabilities. The contributions my parents made can also not be understated. From supporting me through tough times to, more obviously, financing this whole operation, I am exceptionally grateful for everything they have done for me. In particular, I would like to thank my father, who read and corrected more than 60 pages about some fungus he did not even know existed and is not particularly interested in. I can assure you this effort, and all the other efforts made by family members, did not go unnoticed.

Finally, I would like to thank all of my friends. The people I hang around with have not changed for over 10 years, and I would not have it any other way. I know I have a group of people in my corner who I can always count on when I need to.

With all of that being said, I hope you find this thesis to be an interesting and pleasant read.

Raf Verdoodt

Abstract

Rhizoctonia solani is a soilborne pathogen affecting production of a wide range of crops, including faba bean (*Vicia faba* L.). Based on hyphal anastomosis, isolates are classified into anastomosis groups (AGs). Previous studies based on rDNA-ITS gene region analysis suggest AG-5 can be divided into two subgroups. This study supports these findings, based on anastomosis reactions between isolates of AG-5. When the pathogenicity and aggressiveness of different AGs on faba bean was evaluated, it was found that AG-3 was the least pathogenic. In general, isolates from AG-4 HGII and AG-11 were the most aggressive, although results varied. *Bacillus velezensis* strain GA1, *Pseudomonas fuscovaginae* strain UPB0736 and *Pseudomonas asplenii* G1 strain RHF3.3-3 were all observed to substantially inhibit the growth of *R. solani in vitro* while *Pseudomonas* U2 strain COR58 did not, with results varying depending on incubation temperature and *R. solani* isolate. RHF3.3-3 and GA1 were not very effective in reducing *R. solani* symptoms on faba bean. Although GA1 did seem to reduce symptoms, RHF3.3-3 appeared to increase disease severity. GA1 also improved the emergence of the plants while RHF3.3-3 impaired emergence. Fludioxonil, while also improving emergence, again did not appear to reduce disease severity *in vivo*. The EC_{50} of fludioxonil was *in vitro* determined to be around 0.10 mg/L for most isolates, with some exceptions. Finally, the growth inhibition of *R. solani* caused by GA1 mutants producing only one cyclic lipopeptide was examined. It was found that iturin is likely the most important for the antifungal activity of GA1.

Samenvatting

Rhizoctonia solani is een bodemgebonden pathogeen dat de productie van diverse gewassen beïnvloedt, waaronder faba boon (*Vicia faba* L.). Op basis van hyfale anastomose worden isolaten ingedeeld in anastomosegroepen (AG's). Eerdere studies gebaseerd op analyse van de rDNA-ITS genenregio suggereren dat AG-5 kan onderverdeeld worden in twee subgroepen. Dit onderzoek ondersteunt deze bevindingen gebaseerd op anastomosereacties tussen isolaten van AG-5. Wanneer de pathogeniciteit en agressiviteit van verschillende AG's op faba boon werden geëvalueerd, werd AG-3 als minst pathogeen gevonden. Algemeen waren isolaten van AG-4 HGII en AG-11 het meest agressief, alhoewel resultaten varieerden. *Bacillus velezensis* stam GA1, *Pseudomonas fuscovaginae* stam UPB0736 en *Pseudomonas asplenii* stam G1 RHF3.3-3 inhibeerden allemaal aanzienlijk de groei van *R. solani in vitro*, terwijl *Pseudomonas* U2 stam COR58 dat niet deed. Deze resultaten varieerden afhankelijk van incubatietemperatuur en *R. solani* isolaat. RHF3.3-3 en GA1 waren niet erg doeltreffend in het reduceren van *R. solani* symptomen op faba boon. Hoewel GA1 enige vermindering van symptomen leek te veroorzaken, leek RHF3.3-3 ziekte ernstiger te maken. GA1 verbeterde ook de opkomst van de planten, terwijl RHF3.3-3 de opkomst belemmerde. Fludioxonil, hoewel het ook de opkomst verbeterde, verminderde opnieuw niet de ernst van de ziekte *in vivo*. De EC_{50} van fludioxonil werd *in vitro* bepaald rond 0.10 mg/L te zijn voor de meeste isolaten, met enkele uitzonderingen. Ten slotte werd de groei inhibitie van *R. solani* door GA1-mutanten die slechts één cyclische lipopeptide produceerden onderzocht. Hierbij werd vastgesteld dat iturine waarschijnlijk het belangrijkste is voor de antifungale activiteit van GA1.

Table of contents

List of abbreviations

AG	Anastomosis group
ANOVA	Analysis of variance
B.	*Bacillus*
BI	Bridging isolates
CFU	Colony Forming Unit
CLiP	Cyclic lipopeptide
DSI	Disease severity index
EC_{50}	Half maximal effective concentration
GI	Growth Inhibition
ITS	Internal transcribed spacer
KB	Kings' B
LB	Luria-Bertani
OD	Optical density
P.	*Pseudomonas*
PAI	Percentage Area Inhibition
PDA	Potato dextrose agar
R.	*Rhizoctonia*
S.	*Stachybotrys*
Spp.	Species
T.	*Trichoderma*
V.	*Verticillium*
VCP	Vegetatively compatible population

1) Introduction

Rhizoctonia solani is a soilborne pathogen causing significant damage to a wide range of economically important crops (Ajayi-Oyetunde & Bradley, 2018). The classification of different isolates from this fungus is based on the capability of hyphae to fuse with one another, named (hyphal) anastomosis (Ajayi-Oyetunde & Bradley, 2018). Isolates capable of anastomosis are classified as belonging to the same anastomosis group (AG) (Ajayi-Oyetunde & Bradley, 2018; Carling, 1996). From an agricultural standpoint this classification is important as *R. solani* isolates from the same AG tend to cause similar symptoms on similar host plants (Agrios, 2005). Faba bean (*Vicia faba* L.) is one crop of which the production is significantly affected by *R. solani* (Assunção et al., 2011; Baudoin et al., 2006; Infantino et al., 2006; Salt, 1982). Faba bean is cultivated for its use as feed and break crop, as well as being a staple food in the Mediterranean and many parts of Asia (Crépon et al., 2010; Kirk, 2004). Different AGs of *R. solani* cause different symptoms on faba bean, one of the most common being root rot.

In order to mitigate the production losses due to *R. solani,* many different disease control methods have already been developed including the use of good agricultural practices, fungicides and resistant cultivars. The shortcomings of these control methods have led to the research and development of biological control as an alternative (Aydin, 2015; Hua, 2014). Some of the most extensively studied organisms for their use as biological control agents are *Pseudomonas* and *Bacillus* spp.. These bacteria persist in plant roots where they suppress *R. solani* by competing for nutrients, secreting antifungal metabolites and mucolytic enzymes, and providing systematic resistance to the plant (Aydin, 2022; Weller, 1988; Yin et al., 2013). Both genera are capable of producing cyclic lipopeptides (CLiPs) which are compounds with many properties interesting for their use in biological control of not only *R. solani,* but also other plant diseases. (Bender et al., 1999; Cesa-Luna et al., 2023; Fan et al., 2017; Fira et al., 2018; Raaijmakers et al., 2006).

This thesis entails several different topics and research objectives related to *R. solani* and its biological control. Phylogenetic studies based on the ribosomal DNA internal transcribed spacers (rDNA-ITS) gene region suggest that AG-5 can be divided into two new subgroups, namely AG-5-1 and AG-5-2 (Marcou, unpublished). This subdivision of AG-5 into two subgroups was further researched using anastomosis reactions. The pathogenicity and aggressiveness of different isolates and AGs was also determined. Finally, the biocontrol activity of CLiPs and CLiP producing bacteria was evaluated and compared to a chemical control method using fludioxonil seed coating.

2) Literature review

2.1) *Rhizoctonia* spp.

Members of the genus *Rhizoctonia* are heterogeneous, filamentous fungi that do not produce asexual spores (mitospores) and share some common features in their anamorphic (asexual) state (González García et al., 2006). Most of these fungi are root-associated soilborne pathogens, although saprophytic and symbiotic taxa have also been observed (González García et al., 2006). When the original genus concept, established by de Candolle (1815), was reviewed by Parmeter & Whitney (1970), they concluded that the basic characteristics of *Rhizoctonia* came down to the production of survival structures called sclerotia that have a uniform texture, and mycelium that is associated with roots of living plants. Next to the lack of production of mitospores also other characteristics such as brown pigmented hyphae and hyphal constrictions at branch points forming right angles were used to classify fungi into the genus *Rhizoctonia* (González García et al., 2006).

2.1.1) Taxonomy of *Rhizoctonia* spp.

In the past, fungi were classified within the anamorphic genera *Rhizoctonia,* while the teleomorph (sexual state) of the same species were classified as belonging to a different family and order leading to multiple scientific names for the same fungi (Ajayi-Oyetunde & Bradley, 2018). This, combined with the facts that some anamorphs do not have a teleomorph assigned to them and that an anamorph has not been determined for some teleomorphs, leads to a complicated taxonomy (González García et al., 2006).

Due to the complexity of this genus, efforts have been made to clear up the taxonomy. The genus *Rhizoctonia* is composed of species differing in nuclear state that all can cause severe damage to many plants, although with varying levels of pathogenicity (Li et al., 2021). Based upon this nuclear state the genus can be classified taxonomically in three major groups: the multinucleate *Rhizoctonia* (teleomorphs *Thanatephorus* and *Waitea*), the binucleate *Rhizoctonia* (teleomorphs *Ceratobasidium* and *Tulasnella*) and the uninucleate *Rhizoctonia* (teleomorph *Ceratobasidium*) (Sharon et al., 2006). Fusion of vegetative hyphae is called (hyphal) anastomosis, which is used to classify isolates of *Rhizoctonia* (Ajayi-Oyetunde & Bradley, 2018). Isolates that are capable of anastomosis are classified as belonging to the same anastomosis group (AG) (Ajayi-Oyetunde & Bradley, 2018; Carling, 1996). Classification of *Rhizoctonia* species (spp.) is mainly based on characterization of the aforementioned nuclear condition and anastomosis grouping (González García et al., 2006; Sneh et al., 1991). When teleomorphic stages are available, studies of the morphology of basidia and basidiospores (sexual spores) have also been employed (González García et al., 2006). More recently, molecular techniques such as DNA sequencing of ribosomal RNA genes and biochemical methods such as isozyme and fatty acid analysis amongst others have also been used in an effort to classify *Rhizoctonia* spp. (Sharon et al., 2006).

Depending on the author, *Rhizoctonia* is divided into seven or eight anamorphic genera (González García et al., 2006). In this thesis a closer look is taken at the species *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris* Frank (Donk)), as it is the most studied species of the genus *Rhizoctonia* (González García et al., 2006) and the subject of this thesis.

2.2) *Rhizoctonia solani*

Rhizoctonia solani is a soilborne necrotroph that causes significant damage to a wide range of economically important crops (Ajayi-Oyetunde & Bradley, 2018). These crops range from grains such as wheat, rice, and maize (Ajayi-Oyetunde & Bradley, 2018), to legumes such as soybean, peanut and faba bean (Ajayi-Oyetunde & Bradley, 2018; Akladious et al., 2019), other vegetables such as tomato and carrot (Gondal et al., 2019; Mori et al., 2021) and many more. *Rhizoctonia solani* can cause a multitude of symptoms depending on the specific *R. solani* isolate, the AG it belongs to and the host plant (Ajayi-Oyetunde & Bradley, 2018). Symptoms can consist of rotting of the seeds, roots, hypocotyl, crown, stem, limbs and pods along with black scurf, stem cankers, seedling blight and pre- and post-emergence damping-off (Ajayi-Oyetunde & Bradley, 2018). Which of these symptoms occur not only depends on the host plant, but also on the growth stage of the plant and on the environmental conditions (Agrios, 2005). Usually, *R. solani* has a rapid growth rate and is pathogenic, although this may be lacking in some isolates (Sneh et al., 1991).

2.2.1) Morphology

Due to *Rhizoctonia* spp. not producing asexual spores, these fungi primarily exist in nature as vegetative hyphae and sclerotia (Hua, 2014). Three different types of hyphae are produced: straight and non-infecting runner hyphae, short swollen lobate hyphae responsible for the formation of appressoria or dome-shaped infection cushions and specialized hyphae involved in the formation of sclerotia (Hua, 2014; Misra et al., 1994; Sharma, 2004; Sneh et al., 1991). Mature hyphae are uniform and rigid with branches arising at right (90°) or acute (45°) angles from the main branch (Butler & Bracker, 1970; Duggar, 1915; Sneh et al., 1991). Young hyphae are hyaline but change in color when maturing due to the accumulation of melanin in the cell walls, first becoming yellowish and then brown (Duggar, 1915; Kotila, 1947; Saksena & Vaartaja, 1961; Sneh et al., 1991). An illustration of the hyphae of *R. solani* is given in Figure 1.

Sclerotia usually are composed of compact masses of monilioid cells, which are simple branched chains of cells also shown in Figure 1 (Misra et al., 1994; Sneh et al., 1991). This is not always the case, as sometimes sclerotia are made from undifferentiated hyphae (Butler & Bracker, 1970). Sclerotia are survival structures due to the presence of melanin in their thick cell walls. This leads to sclerotia being a primary source of *Rhizoctonia* inoculum (Keijer, 1996; Misra et al., 1994).

Under certain environmental conditions such as high humidity, isolates of *Rhizoctonia* spp. can sporulate giving rise to teleomorphic structures which appear on soil, leaves and infected stems just above the ground (Agrios, 2005). Basidia are formed on dense interwoven mats of short hyphal branches and may be cylindrical, oval or spherical. Sterigmata that hold the basidiospores, arise from these basidia, varying in number from one to seven but usually being four (Sneh et al., 1991). Basidiospores are hyaline, thin walled, smooth, not amyloid structures that are typically uninucleate (Stalpers & Anderson, 1996) and can also vary in shape being spherical, oval, or pyriform (Sneh et al., 1991). An illustration of the basidia is given in Figure 3. These characteristics of the teleomorph are important for classification of *Rhizoctonia* spp. (Sneh et al., 1991).

Based on the works of Duggar (1915) and Parmeter & Whitney (1970) defining characteristics of *R. solani* isolates can be listed as being I) brown coloration in the mature hyphae, II) branching near the distal septum of cells in young vegetative hyphae, III) constriction of hyphae at the branching points, IV) formation of septa a short distance away from the point of origin of hyphal branches, V) dolipore

septa that permits unrestricted movement of cytoplasm, mitochondria and nuclei from cell to cell and VI) multinucleate cells in young vegetative hyphae (Ajayi-Oyetunde & Bradley, 2018; Sneh et al., 1991). Additional characteristics are usually present but may be lacking in some isolates. Such morphological characteristics are monilioid cells and sclerotia of uniform texture and hyphae greater than 5 µm in diameter (Sneh et al., 1991). Some features are never present such as clamp connections, conidia, sclerotia differentiated into rind and medulla, rhizomorphs and pigmentation differing from brown (Sneh et al., 1991).

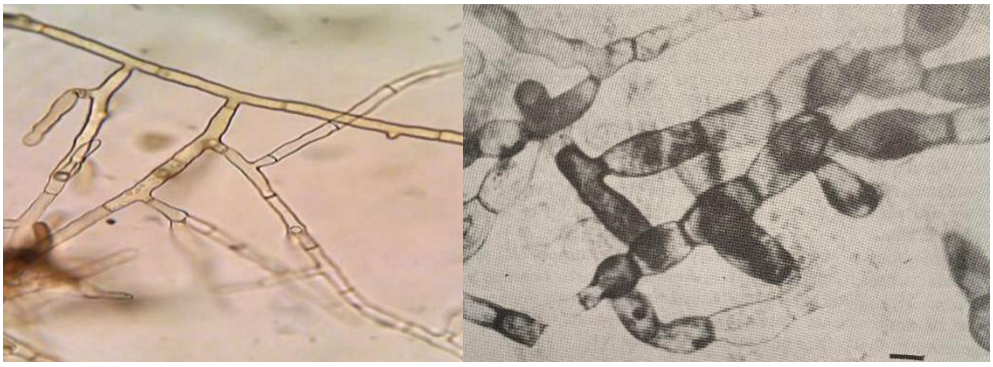


Figure 1. (left) Hyphae of *Rhizoctonia solani* (Nagaraj et al., 2019) and (right) monilioid cells of *Rhizoctonia solani* (Sneh et al., 1991).

2.2.2) Anastomosis grouping

As mentioned before, classification of *Rhizoctonia* isolates is based on hyphal anastomosis (Ajayi-Oyetunde & Bradley, 2018; Carling, 1996) and is considered as a powerful tool to study the *Rhizoctonia* species' complex taxonomy (Carling, 1996). Based on this system, genetically similar *R. solani* isolates are grouped together belonging to the same AG (Ajayi-Oyetunde & Bradley, 2018; Carling, 1996). This does not mean that isolates belonging to the same AG are therefore similar in behavior and appearance (Carling, 1996). There do tend to be differences to grouping based on pathogenicity, colony morphology or other physical features (Carling, 1996). Even though AGs are not entirely host specific, there is a tendency for isolates of the same AG to cause similar symptoms on the same plants (Agrios, 2005).

When studying anastomosis, it is necessary to analyze the cytological reactions in the "zone of confrontation" being the area where hyphae of two isolates can come into contact with one another (Carling, 1996). Over the years, different terminologies have been used in order to categorize different types of anastomosis reactions (Carling, 1996). Unfortunately, certain cytological details are lacking which are needed to understand the criteria used to define each category (Carling, 1996). That is why (Carling et al., 1988) created a system of four categories, ranging from C3 to C0 anastomosis reactions, in an attempt to overcome problems previous classifications had. Table 1 gives a description of these categories and what they mean for the relationship between isolates. An example of each reaction is given in Figure 2. As is the case for C2 and sometimes C1 reactions, fusing- and adjacent hyphal cells die when anastomosis occurs making it easy to identify the anastomosis reactions (Agrios, 2005; Carling, 1996). *Rhizoctonia solani* can also be divided into smaller, more homogeneous subunits on the basis of C3 reactions that indicate a closer level of relatedness than either C1 or C2 reactions do (Carling, 1996). While first inaccurately being called "clones", these subunits where later renamed to vegetatively compatible populations (VCPs) based on a compatible vegetative reaction, being the C3 reaction, without implying the isolates being genetically identical (Carling, 1996).

Table 1. Categories of anastomosis reaction in *Rhizoctonia solani* (Carling et al., 1988).

Category	Relatedness	Relation between isolates	Description of hyphal interaction
C3	Closely related	Same AG[1] Same VCP[2]	Cell walls and membranes fuse; Diameter of anastomosis point (nearly) equal to diameter of hyphae; Anastomosing cells generally do not die; Anastomosis point frequently not obvious
C2	Related	Same AG Different VCP	Connection between cell walls but connection between membranes is uncertain; Diameter of anastomosis point is smaller than diameter of hyphae; Anastomosing and adjacent cells always die; Obvious anastomosis point
C1	Distantly related	Same or different AG	Contact between hyphae; Apparent cell wall connection but no evidence of cell wall penetration or membrane connection; Occasionally one or both anastomosing and adjacent cells die
C0	Not related	Different AG	No interaction

[1] Anastomosis group; [2] Vegetatively compatible populations

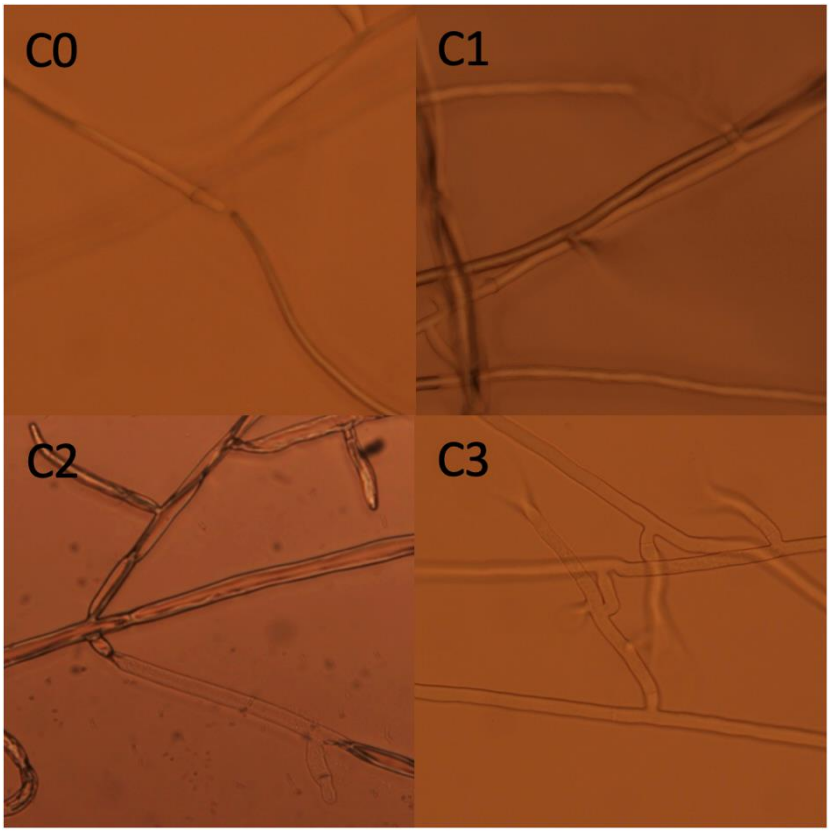


Figure 2. Example of the types of anastomosis reactions in *Rhizoctonia solani*.

There have currently been 14 AGs identified in *R. solani*, with some subdivision of these groups based on characteristics other than anastomosis or, in the case of AG-2, the frequency of anastomosis (Ajayi-Oyetunde & Bradley, 2018). Members of groups AG-1 to AG-13 are generally only capable of anastomosis amongst themselves while the isolates belonging to AG-BI (bridging isolates) are also capable of anastomosis with isolates belonging to other AG (Chosdon et al., 2021), generally resulting in a C1 reaction (Carling, 1996). There is some disagreement on this classification, as some authors see AG-BI as a subgroup of AG-2 (Lübeck, 2004). The existence of bridging isolates shows that the separation into AGs does not mean that the members from different AGs are genetically isolated from one another (Carling, 1996). The following is a list of all AG in *R. solani* together with their respective subgroups (Ajayi-Oyetunde & Bradley, 2018):

- AG-1 (IA, IB, IC, ID, IE, IF)
- AG-2 (1, t, Nt, 2IIIB, 2IV, 2LP, 3, 4)
- AG-3 (TB, PT, TM)
- AG-4 (HGI, HGII, HGIII)
- AG-5
- AG-6 (HG-I, GV)
- AG-7
- AG-8 (1, 2, 4, 5)
- AG-9 (TP, TX)
- AG-10
- AG-11
- AG-12
- AG-13
- AG-BI

2.2.3) Disease cycle

The disease cycle of *R. solani* is shown in Figure 3. The inoculum of disease in plants can be sclerotia, mycelium or basidiospores (Keijer, 1996) and are dispersed by rain, irrigation or flood water and with tools and anything else carrying contaminated soil (Agrios, 2005). While more fragile than sclerotia and mycelium, basidiospores still play an important role in the genetic variation and long-distance dispersal of *R. solani* (Keijer, 1996). Sclerotia, present in the soil or in plant residues, are the primary survival structures whilst mycelium can also be useful for dispersal and survival of *R. solani* by rapidly growing in soils and being persistent on plant debris (Höfte, 2021; Keijer, 1996; Papavizas G. C., 1970; Papavizas & Davey, 1962). Sclerotia and mycelium can also be present in contaminated seeds (Agrios, 2005).

Sclerotia first need to germinate and form mycelium in order to infect the plant (Keijer, 1996). This mycelium then follows the same infection process as free mycelium does, by first colonizing the plant surface and aggregating to form infection cushions (Höfte, 2021). From these infection cushions, mycelium can penetrate the host plant, either directly through the cuticle or through the junction of anticlinal walls of epidermal cells (Weinhold & Sinclair, 1996; Yang et al., 1992), where toxins and lytic enzymes are produced (Höfte, 2021). Infection results in a number of negative responses in the host plant such as collapsing of invaded cells, plasmolysis of adjacent epidermal cells, destruction of cellular organelles, cell wall swelling and more (Weinhold & Sinclair, 1996; Yang et al., 1992). Infections are worse when the soil is moderately wet in comparison to dry or waterlogged soils. This is also the case

if young plants are infected when plant growth is slow due to the adverse environmental conditions for the plant (Agrios, 2005).

Infection through basidiospores is slightly different since these infect the aerial parts of the host plants rather than the roots (Naito, 1996). The germinating basidiospores form appressoria that can directly penetrate into the epidermal cells and form a stroma-like structure within the epidermal cells or the upper layer of the mesophile cells (Naito, 1996). Out of these stroma-like structures, hyphae grow that cause primary lesions on the aerial parts of the host plants (Naito, 1996). Whilst sclerotia or mycelium are more persistent and thus cause more infections (Hua, 2014; Keijer, 1996), with favorable environmental conditions, infection by basidiospores is a significant danger for aerial parts of certain host plants (Naito, 1996).

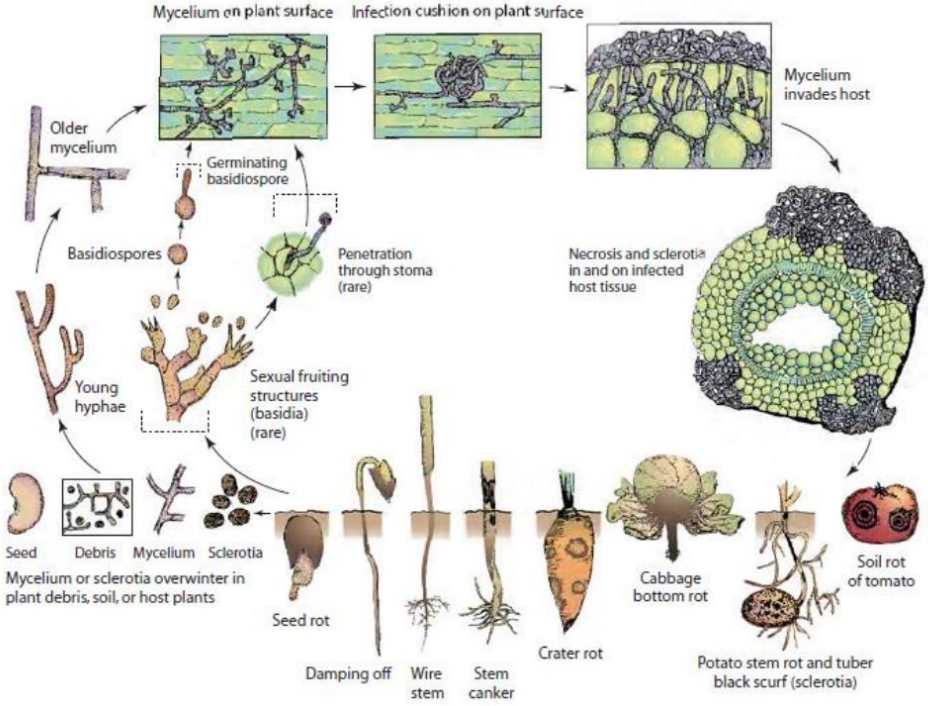


Figure 3. Disease cycle of *Rhizoctonia solani* (Agrios, 2005)

2.3) *Vicia faba* L.

2.3.1) General usage

Faba bean (*Vicia faba* L.), also commonly referred to as broad bean, field bean, fava bean or horse bean, is a leguminous crop mainly grown for its use as feed and food, but also as a break crop due to its use as a green manure and the production of silage (Kirk, 2004). Humans consume faba bean as mature dry beans, green vegetables, or processed food while dry seeds, green haulm and dry straw are used as animal feed (Kumari & Makkouk, 2007). It has long been a staple food in the Mediterranean and continental areas like Southern China, Northwest India, Iraq, Iran, Syria, and Pakistan (Crépon et al., 2010). The seeds are considered to have a good nutritional value due to the high protein and energy content, but anti-nutritional effects have been observed in both animals and humans (Crépon et al., 2010). Such is the case for people with a glucose-6-phosphate dehydrogenase enzyme deficiency, who are at risk to favism, a condition associated with hemolytic anemia, when consuming faba beans (Prabhu & Rajeswari, 2018). The seeds are also believed to have certain

medicinal properties, possibly having antioxidant, anti-fungal, anti-diabetic and anticancer activities while also combating Parkinson's disease and being resistant to human cytomegalovirus (Kirk, 2004; Prabhu & Rajeswari, 2018).

2.3.2) Morphology

Morphologically, the plant can be described as an erect, unbranched annual herb with square and hollow stems which grows up to 1-2 m (Kirk, 2004). The plant has alternate, pinnate leaves with two to six leaflets (Kirk, 2004). Short racemes in the leaf axis usually hold one to eight flowers each with the oldest flower close to the base and the youngest to the apex of the racemes (Kirk, 2004). The flowers are pollinated through bees which is why they are scented and nectar is produced (Kirk, 2004).

2.3.3) Cultivation

Faba beans have been cultivated for thousands of years across many different environments which together with mating systems and human selection has led to large variety in cultivars (Kirk, 2004; Maalouf et al., 2013). Since faba bean is able to germinate at relatively low temperatures it can be sown as early or as late as the soil allows (Neuvel, 1991; Sharan et al., 2021). For summer varieties, this means sowing ideally happens from the middle of February to the end of March (de Meulemeester, 2015; Neuvel, 1991) while for winter varieties sowing ideally happens from mid-October to mid-November (van den Broeck, 2017). Heavy soils, being sandy loam or clay soils, are ideal but lighter soils can also be used as long as the pH_{KCL} value of the soil is not below 5.5, in order not to jeopardize the root development and drought resistance of the plants (de Meulemeester, 2015; Neuvel, 1991; van den Broeck, 2017). A good aeration of the soil together with a pH value above 6 is also necessary to ensure the development of *Rhizobium* bacteria needed for nitrogen fixation (de Meulemeester, 2015; van den Broeck, 2017). Beans which are destined for the vegetable market are sown at a density of around 8-13 plants/m² while beans meant for industrial purposes are sown at a higher density of around 13-18 plants/m² (Neuvel, 1991), ideally on an even but not too fine sowing bed (de Meulemeester, 2015; van den Broeck, 2017). Winter varieties need to be sown slightly deeper (around 7 cm) than summer varieties (around 4-5 cm) in order to withstand frost damage (de Meulemeester, 2015; van den Broeck, 2017). Because of the symbiose with *Rhizobium*, being able to fixate anywhere up to 250 kg N/ha, faba bean plants generally do not need to be fertilized with nitrogen, but a small amount of around 40-60 kg N/ha can be given at the start of the growing season (de Meulemeester, 2015; Neuvel, 1991; van den Broeck, 2017). A fertilization of 40-55 kg P_2O_5/ha and 75 kg K_2O is recommended (de Meulemeester, 2015; van den Broeck, 2017). The time of harvesting depends on the hardness of the seeds which is in turn determined by the cultivar used and, if the seeds are processed, by the industrial demand (Neuvel, 1991). For summer varieties, harvesting usually takes place from mid-August to the beginning of September while for winter varieties harvesting usually takes place slightly earlier from the end of July to the beginning of August (de Meulemeester, 2015; van den Broeck, 2017).

2.3.4) World production

Faba bean is cultivated all over the world due to its ability to adapt to a wide range of climate and soil conditions (Crépon et al., 2010; Singh et al., 2013). An overview of the production in 2021 in the major

faba bean producing regions is given in Table 2. Worldwide about 1.7 million tons of green faba beans and about 6 million tons of dry faba beans were harvested in 2021 with Algeria being the leading producer of green faba beans and China being the leading producer of dry faba beans (FAOSTAT, 2023). Table 2 also indicates that the production of dry faba beans is relatively higher in industrialized regions such as European countries and China while the production of green faba beans is relatively higher in developing countries such as in Africa. This is to be expected, since industrialized nations mainly use faba bean as a feed while developing nations use it as food (Akladious et al., 2019).

Table 2. Area harvested, yield per hectare and total yield of green and dry faba beans in 2021 in the major faba bean producing regions of the world. The ten largest countries in green faba been production as well as in dry faba bean production are given. "/" indicates that there is no available data (FAOSTAT, 2023).

Region/ country	**Green faba bean**				**Dry faba bean**			
	Area harvested (1000 ha)	Yield (tons /ha)	Total yield (1000 tons)	World ranking in total yield	Area harvested (1000 ha)	Yield (tons /ha)	Total yield (1000 tons)	World ranking in total yield
World	277.5	6.22	1725.4		2722.7	2.19	5964.4	
Africa	75.2	8.96	674.1		758.9	2.14	1620.4	
Algeria	32.3	8.58	277.4	1	37.4	1.06	39.6	19
Egypt	20.7	9.21	190.9	2	26.4	3.98	105.1	11
Ethiopia	1.6	7.25	11.5	27	498.8	2.18	1089.5	2
Morocco	8.4	8.62	72.3	8	104.9	1.25	131.2	9
Tunisia	6.2	13.47	83.6	5	54.7	1.39	76.0	15
Sudan	/	/	/	/	34.2	5.17	176.7	7
Asia	40.8	10.50	428.5		846.4	2.10	1779.0	
China	13.7	13.67	187.2	3	804.3	2.10	1690.6	1
Syria	9.3	7.46	69.6	9	15.3	1.58	24.2	23
Europe	42.9	6.12	262.6		668.4	2.74	1832.1	
France	6.5	5.02	32.5	18	78.0	2.36	184.2	6
Germany	0.4	6.32	2.3	37	57.6	4.10	235.9	5
UK	5.1	5.28	26.9	21	187.6	3.7	694.2	3
Italy	7.4	5.51	40.5	14	62.5	1.90	118.6	10
Spain	6.2	8.48	52.1	10	21.9	1.12	24.4	
Poland	14.7	6.02	88.5	4	35.9	2.71	97.4	12
Lithuania	0.1	1.83	0.1	51	76.2	1.79	136.4	8
North America	15.8	2.77	43.7		/	/	/	
Central America	45.2	2.48	112.2		45.5	1.43	64.84	
Mexico	12.9	6.39	82.4	6	23.4	1.61	37.6	20
South America	57.4	3.55	203.7		125.6	1.17	146.4	
Bolivia	26.8	1.53	41.0	13	14.3	0.91	13.1	31
Peru	13.5	5.67	76.8	7	52.6	1.57	82.4	13
Oceania	0.1	8.65	0.6		269.0	1.90	509.8	
Australia	/	/	/	/	269.0	1.90	509.8	4

2.4) Disease caused by *Rhizoctonia solani* on *Vicia faba* L.

2.4.1) Impact on production

The production of faba bean is significantly affected by plant pathogens, and in particular *R. solani*, being the main root disease of faba bean in many countries (Assunção et al., 2011; Baudoin et al., 2006; Infantino et al., 2006; Salt, 1982). Rhizoctonia diseases are mostly problematic for seedlings and young plants, whereas older plants are less prone to damage (Abawi, 1989; Agrios, 2005). Little research has been conducted on the effects and production losses of faba bean due to *R. solani*. However, infection can affect crop production significantly, as it does in similar bean crops (Assunção et al., 2011; Baker, 1970; Tu et al., 1996). Bean yield losses due to root diseases are considerable and can vary from field to field and season to season (Abawi, 1989). Furthermore, root diseases can cause a reduced nutrient uptake efficiency in bean plants which leads to plants more susceptible to environmental stressors (Abawi, 1989). The optimum temperature for *R. solani* infection is 15-18°C and disease incidence is higher in moderately wet soils in comparison to waterlogged or dry soils (Agrios, 2005).

2.4.2) Symptomology

R. solani is the cause of many symptoms on faba bean being rotting of the roots, seed, collar, crown and stem, seedling blight, stunting, wilt, cankers, stem lesions, and pre- and post-emergence damping-off (Assunção et al., 2011; Azimi et al., 2005; Engelkes & Windels, 1996; Mahmoud et al., 2007; Mwiindilila, 1984; Paul et al., 2022; Rashid & Bernier, 1993; H. Yu et al., 2022). Different AGs of *R. solani* cause these symptoms in different parts of the world. A summary is given in Table 3. While symptoms like seedling blight and root rot tend to occur in more temperate areas, in humid tropics *R. solani* can also infect aerial parts of other leguminous crops inciting 'web blight' (Abawi, 1989; Tu et al., 1996).

As root rot is one of the more common symptoms on faba bean and also what will be evaluated in this thesis to assess disease severity, a closer look at the symptom is given here. Figure 4 shows root rot on Phaseolus bean (*Phaseolus vulgaris* L.) and faba bean together with another common symptom of *R. solani,* being seedling blight, on soybean. Characteristic of *R. solani* infections are reddish brown, sunken lesions on the stem, hypocotyl and taproot (Abawi, 1989). On young seedlings these lesions expand quickly and result in damping-off while on older plants the lesions can join together and girdle the stem which hampers the growth and may eventually kill the plant (Abawi, 1989). In general, the result of root rot is a reduced health of the plant with discoloration and rotting of the root as well as the stem (Abawi, 1989).

Table 3. Anastomosis groups (AGs) of *Rhizoctonia solani* associated with faba bean.

AG/subgroup	Symptoms	Geographic origin	Reference
AG-1-IB	Root rot, crown rot	Iran	Azimi et al., 2005
AG-2-1	Root rot	Canada	Yu et al., 2022
	Deep, black stem lesions	Canada	Mwiindilila, 1984
AG-2-2	Root rot	Canada	Yu et al., 2022
	Seed rot, seedling blight	Canada	Rashid & Bernier, 1993
	Deep, black stem lesions	Canada	Mwiindilila, 1984
AG-2-2IIIB	Collar rot, root rot	Bangladesh	Paul et al., 2022
	Root rot, crown rot	Germany	Boine et al., 2014
	Stem rot	USA	Engelkes & Windels, 1996
AG-2-2IV	Stem rot	USA	Engelkes & Windels, 1996
AG-3	Reduced plant growth and nodulation	Tunisia	Djébali et al., 2014
	Deep, black stem lesions	Canada	Mwiindilila, 1984
AG-4	Seed rot, seedling blight	Canada	Rashid & Bernier, 1993
	Canker	Brazil	Assunção et al., 2011
	Root rot	Egypt	Akladious et al., 2019; Mohamed et al., 2014b, 2014a, 2015
		Canada	Yu et al., 2022
	Root rot, crown rot	Iran	Azimi et al., 2005
	Deep, black stem lesions	Canada	Mwiindilila, 1984
AG-5	Seed rot, seedling blight	Canada	Rashid & Bernier, 1993
	Root rot	Canada	Yu et al., 2022
AG-7	Root rot, crown rot	Iran	Azimi et al., 2005
Unknown	Damping-off, root rot/wilt	Egypt	Abdel-Monaim, 2013
	Seed rot, wilt, stunting, pre- and post-emergence damping-off	Egypt	Mahmoud et al., 2007

Figure 4. Root rot of Phaseolus bean (Schwartz, 2008) (top left) and faba bean (Credits: Mariann Wikström, Agro Plantarum AB) (bottom) and seedling blight of soybean caused by *Rhizoctonia solani* (Mueller, 2021).

2.5) Disease control of *Rhizoctonia solani*

An integrated disease management system where multiple measures are taken to reduce the damage to the crop is important when managing *R. solani* infections (Ajayi-Oyetunde & Bradley, 2018). In fact, the incidence and severity of root disease can even increase due to improper agricultural techniques such as a continuous bean production system without a proper crop rotation and soil compaction (Abawi, 1989). In the following sections, an overview is given of some possible cultural and chemical control measures as well as resistance breeding and biological control methods carried out to manage *R. solani*, specifically on faba bean.

2.5.1) Cultural control

Several agricultural practices can be implemented to reduce the incidence of *R. solani* in the field. The first is proper crop rotation. Monocropping beans increases the amount of inoculum in the soil, which leads to a higher incidence of *R. solani* (Abawi, 1989). While *Rhizoctonia* spp. have a wide host range, individual AGs and subgroups generally have a more limited number of crops they can infect (Butler,

1993; Van Bruggen et al., 1996). Since multiple AGs, typically varying in virulence to a host, are present in the soil, a well thought out crop rotation might shift the presence of these different AGs in the soil towards isolates which are less virulent to a certain host (Herr, 1993). While completely eradicating *R. solani* from the soil is difficult, diversifying the crop rotation with non-host plants like oats, barley, wheat and maize works best for pathogen activity while inoculum levels remain relatively high in crop rotations with other beans, peas, or potatoes (Abawi, 1989; Burke & Kraft, 1973). It should be noted that there are multiple isolates that have the ability to infect a wide array of crops making *R. solani* persist in the soil and necessitating the use of other management strategies in combination with crop rotation (Ajayi-Oyetunde & Bradley, 2018).

Soil amendments are another way to control Rhizoctonia diseases. Organic amendments for example being compost, cover crops or manure, could have a positive impact but also a negative impact depending on the material and state of decomposition (Van Bruggen et al., 1996). Agrios (2005) recommends mulching fields with certain plant materials or covering fields with photodegradable plastic. Huber & Sumner (1996) point to the positive effects of organic amendments such as the incorporation of certain crops or crop residues, manure, food processing wastes, composts, sewage sludge and chitin while also noting the value inorganic amendments, like specific nutrients, can have.

There are conflicting results when it comes to the tillage system applied. On the one hand, minimum or no-tillage systems can lead to an increase in the occurrence of disease due to *R. solani* inoculum remaining near the soil surface (Van Bruggen et al., 1996). In this case deep plowing up to 20-25 cm can reduce disease incidence by reducing the inoculum load near the seeds (Abawi, 1989; Ajayi-Oyetunde & Bradley, 2018; Papavizas & Lewis, 1979). On the other hand, tillage can lead to soil compaction which in turn can lead to a higher severity of root rot (Abawi, 1989; Tu & Tan, 1991; Van Bruggen et al., 1996). No-tillage systems also benefit the population of certain soil fauna like Collembola, which can help reduce disease (Rickerl et al., 1989; Van Bruggen et al., 1996).

A proper seed bed preparation can also aid in controlling diseases caused by *R. solani*. Since the disease is most severe in wet soils, it is important that these areas receive improved drainage or are not used (Agrios, 2005). Seed should be sown on raised seed beds which facilitate good drainage (Abawi, 1989; Agrios, 2005). Seedlings are also exposed to less inoculum and thus have a lower chance of disease when planted at a shallow depth of around 2.5 cm (Abawi, 1989; Leach & Garber, 1970; Manning et al., 1967). Finally, soil disinfestation, either by steaming, fumigation, or solarization can also be used to eradicate inoculum from the soil (Katan, 1996). Because this is expensive, this control mechanism is mostly used for high-value crops such as those grown in greenhouses (Katan, 1996). Furthermore, the use of fumigants like methyl-bromide is often restricted due to negative effects on human health, ozone depletion, loss of soil biodiversity and groundwater contamination (Braun & Supkoff, 1994; Park et al., 2020; Sande et al., 2011). For these reasons, methyl-bromide has been banned in the European Union since 2010 (EUR-Lex, 2021).

2.5.2) Chemical control

The use of fungicides has been the most popular disease control method used by farmers in an effort to minimize yield loss due to *Rhizoctonia* pathogens (Kataria & Gisi, 1996). Fungicides are applied either as a seed or soil treatment or in some cases as a foliar application (Kataria & Gisi, 1996). A wide variety of fungicides with diverse chemical compounds can be used to control *Rhizoctonia* spp. (Kataria & Gisi, 1996), but due to regulations in Belgium as of 2023, only the following three active substances are allowed to be applied onto fields to combat *R. solani* disease on faba bean: azoxystrobin,

fludioxonil, and cyprodinil (Fytoweb, 2023). Fludioxonil can also be used as seed coating to protect the plants (Fytoweb, 2023; Syngenta, 2023).

Chang et al. (2014) found that a seed treatment containing fludioxonil along with the fungicide metalaxyl improved emergence and seed yield for faba bean plants inoculated with *R. solani*. Research from Kataria et al. (2002) showed similar results in trials on Phaseolus bean, with 70-80% of seedlings emerging when seeds coated by fludioxonil where exposed to a *R. solani* isolate belonging to AG-4. The same study found that seed coating with azoxystrobin was less effective with only 56-67% of seedlings emerging (Kataria et al., 2002). Furthermore, Harveson et al. (2005) found no reduction in plant stress or yield improvement when azoxystrobin was applied to Phaseolus bean fields infested with the pathogen. Certain *R. solani* AG-3 isolates which are active on faba bean even show resistance to azoxystrobin (Djébali et al., 2014). So even though scientific literature on the application of these fungicides on faba bean is limited, it seems that a fludioxonil seed coating is the most effective fungicidal treatment to control *R. solani*.

2.5.3) Resistance breeding

Another way to avoid damage by *R. solani* is to use cultivars resistant to the disease (Panella & Ruppel, 1996). Many plant species have cultivars which have resistance to *Rhizoctonia* spp. to varying degrees (Panella & Ruppel, 1996). As immunity to the disease appears to be rare, resistance in this case means that the plant continues to have the ability to grow and function even when the pathogen is present (Panella & Ruppel, 1996). Unfortunately, not many studies have been conducted on *R. solani* resistance of faba bean. Rashid & Bernier (1993) found four non-commercial cultivars with a high level of resistance while Assunção et al. (2011) found another four cultivars. In both studies, the level of resistance also depended on which isolates of *R. solani* the plants where infected with (Assunção et al., 2011; Rashid & Bernier, 1993). Assunção et al. (2011) also found that the inoculum density in the soil and the type of soil also played a role in the resistance of plants to the disease. Further studies need to be carried out in order to assess the viability of resistance breeding of faba bean to combat *R. solani* disease.

2.5.4) Biological control

Because of the saprophytic and soil-borne nature of *R. solani*, the classical control methods listed above can sometimes be insufficient to combat the pathogen (Aydin, 2022). Pesticide usage also comes with adverse effects on human-health and the environment (Hua, 2014). Furthermore, the use of fungicides is hampered by an increasing of amount pathogen resistances in combination with difficult development and legislation surrounding new active substances (Lucas et al., 2015). This has led researchers into finding alternative biological control methods wherein a living microorganism, that does not cause any harm to the plant, suppresses the pathogen (Aydin, 2022; Hua, 2014). In a review by Aydin (2022) on the biocontrol of *R. solani*, it was concluded that the most important antagonistic species of fungi were *Trichoderma* spp., *Gliocladium* spp., *Stachybotrys elegans* and *Verticillium biguttatum,* while the most important antagonistic species of bacteria were *Bacillus*, *Pseudomonas* (in particular *Pseudomonas fluorescens*), *Streptomyces* and *Erwinia* spp.. Aydin (2022) also concluded that there are five antagonistic mechanisms that can aid the plant to combat the pathogen being I) antibiosis (destruction or inhibition of the pathogen due to metabolites, called antibiotics, produced by another organism), II) competition for nutrients and habitat with the

pathogen, III) mycoparasitism, IV) enzymes that disrupt the pathogen cell wall and V) inducing resistance and hypovirulence in the plant (Adams, 1990; Aydin, 2015; Djonović et al., 2007). An antagonist might use one or a combination of these mechanisms (Aydin, 2022). What follows is a discussion of the biocontrol mechanisms and -effectiveness of most important antagonistic species of fungi and bacteria against *R. solani*.

2.5.4.1) Fungi

According to the British Society for Plant Pathology, fungi that live in close association with another fungus while deriving nutrients from this fungus and providing nothing in return are defined as mycoparasites (Van den Boogert, 1996). Mycoparasites can be totally dependent on mycoparasitism to survive, as is the case for *Verticillium biguttatum* (Van den Boogert, 1996). Mycoparasitism can also be used in combination with a saprophytic lifestyle such as in *Trichoderma* and *Gliocladium* spp. (Van den Boogert, 1996).

Trichoderma and *Gliocladium* spp. inhibit radial growth of *R. solani* and exhibit coiling patterns when in contact with the fungus but rarely penetrate the hyphae (Van den Boogert, 1996). Some *Trichoderma* spp. can produce β-(1,3)-glucanase and chitinase, which are able to dissolve hyphae, alongside the ability of these fungi to also produce antibiotics (Aydin, 2022). *Trichoderma* spp. might even be able to induce the production of certain enzymes in faba bean which can further protect the plant from *R. solani* (Hassan et al., 2015). Multiple studies have found *Trichoderma* spp., namely *T. viride*, *T. harzianum*, *T. hamatum*, *T. asperellum*, *T. album*, *T. viride*, *T. virens and T. koningii* to be effective for controlling *R. solani* on faba bean (Abou-Zeid et al., 2003; El-Dabaa et al., 2019; El-Mougy & Abdel-Kader, 2008; Hassan et al., 2015; Hassanein et al., 2006). *Gliocladium virens* has the ability to produce mycotoxins like gliotoxin or gliovirin which can inhibit the growth of *R. solani* (Howell et al., 1993). Research has also found that this species of *Gliocladium* is an effective biocontrol agent for *R. solani* on faba bean (Abou-Zeid et al., 2003; Hassanein et al., 2006).

Contrary to *Gliocladium* spp. and *Trichoderma* spp., *Verticillium biguttatum* cannot inhibit growth extension of *R. solani*, but is able to penetrate hyphae and form internal haustoria-like structures (Van den Boogert, 1996). Also, *V. biguttatum* can produce cell wall dissolving enzymes such as chitinase, glucanase and protease, all the while suppressing the sclerotium development of *R. solani* (Aydin, 2022; Van den Boogert, 1996). The mycoparasite *Stachybotrys elegans* is able to kill both the hyphae and sclerotia of *R. solani* (Charest, 1994). *S. elegans* inhibits the growth of *R. solani*, exhibits coiling patterns and can penetrate the hyphae and grow internally followed by an external sporulation (Charest, 1994). The mycoparasitism of *R. solani* is probably a result of both mechanical and enzymatic processes with enzymatic digestion playing a major role (Charest, 1994). Further research must be conducted on the effectiveness of these mycoparasites in combatting *R. solani* on faba bean.

2.5.4.2) Bacteria

The aforementioned bacteria (*Bacillus, Pseudomonas, Erwinia and Streptomyces* spp.) have several features which aid plants in combatting *R. solani* (Aydin, 2022; Weller, 1988; Yin et al., 2013). These bacteria persist in plant roots and suppress the pathogen by competing for nutrients, secreting antifungal metabolites such as antibiotics or siderophores, secreting mucolytic enzymes and providing systemic resistance to the plants (Aydin, 2015; Weller, 1988; Yin et al., 2013). The most extensively studied bacteria used for biological control of *R. solani* are *Pseudomonas* and *Bacillus* spp.. As these

are also the bacteria that were used as a biological control agent in this thesis, these are discussed further here.

Multiple studies have reported the effectiveness of *Pseudomonas* spp. as a biocontrol agent because of the capability to adapt to the rhizosphere where it has a rapid growth rate and has positive effects on the welfare of plants by suppressing plant diseases and promoting growth and systematic resistance in the plant (Anitha & Das, 2011; Aşkin Şenocak et al., 2019; Aydin, 2022; Couillerot et al., 2009; Dimkić et al., 2022; Saikia et al., 2006). These bacteria can produce several metabolites such as antibiotics, siderophores and several toxins that may hamper the growth of *R. solani* (Aydin, 2022; Saikia et al., 2006). *Bacillus* spp. have similar properties, also having the potential to stimulate systemic resistance and produce antimicrobial compounds (Aydin, 2022; Huang et al., 2012). These bacteria are also capable of protecting plants from abiotic and biotic stress and thus can promote plant growth (Aydin, 2022; Radhakrishnan et al., 2017). Both *Bacillus* spp. and *Pseudomonas* spp. are also able to produce enzymes like chitinases, glucanases and proteases (Dimkić et al., 2022). These aid in combatting fungal pathogens by weakening the cell walls and releasing cell wall components that can be used as a carbon source (Dimkić et al., 2022).

Antimicrobial compounds

Pseudomonas spp. can produce many different compounds interesting for biocontrol (Dimkić et al., 2022), but two particularly compelling metabolites produced by *Pseudomonas* spp. are phenazines and cyclic lipopeptides (CLiPs) (Hua, 2014). Phenazines are tricyclic pigments containing nitrogen, mainly produced by soil inhabitants or plant-associated species (Hua, 2014; Mavrodi et al., 2006). The most noteworthy *Pseudomonas* spp. producing phenazines are *P. fluorescens, P. aeruginosa* and *P. chlororaphis* (Chin-A-Woeng et al., 2003; Gross & Loper, 2009; Hua, 2014). Phenazines are proven to have antibiotic properties with multiple studies already having found that *Pseudomonas* strains that produce the compounds to be effective in combatting *R. solani* (D'aes et al., 2011; Hua & Höfte, 2015; Jaaffar et al., 2017; Laursen & Nielsen, 2004). Phenazines impede microbial growth due to disturbance of the DNA replication and electron transport, loss of membrane functioning, interference in the energy production and the production of superoxide radicals which are fatal to the cell (Chin-A-Woeng et al., 2003; Dimkić et al., 2022; Jacob et al., 2011; Sreejith et al., 2019). Usually, multiple phenazines can be produced by strains of *Pseudomonas,* with the functional groups bound to the aromatic ring in the molecular structure determining the chemical, physical and antibiotic characteristics of the compound (Mavrodi et al., 2006).

Cyclic lipopeptides (CLiPs) are compounds composed of a fatty acid tail coupled to a short oligopeptide, crystalized to form a lactone ring (Raaijmakers et al., 2006). This diverse group of compounds have antimicrobial and cytotoxic properties and are biosurfactants, meaning they are able to lower the surface tension of liquids, which make them interesting for use in biological control (Raaijmakers et al., 2006). Ron & Rosenberg (2001) have proposed four different roles for CLiPs, and biosurfactants in general, being I) pathogenicity, II) antimicrobial activity, III) regulating the attachment to and detachment from surfaces and IV) motility (Raaijmakers et al., 2006). Specifically, CLiP biocontrol relies on direct antibiosis of fungal and bacterial pathogens and stimulating an immune system response in the host plants (Dimkić et al., 2022). Both *Bacillus* spp. (mainly isolates belonging the *B. subtilis* complex) and *Pseudomonas* spp. (isolates belonging to the *P. syringae*, *P. putida* and *P. fluorescens* group) are capable of producing CLiPs (Bender et al., 1999; Cesa-Luna et al., 2023; Fan et al., 2017; Fira et al., 2018).

As of the most recent documentation, CLiPs produced by *Pseudomonas* spp. are classified based on chemical similarities into 14 different groups (Geudens & Martins, 2018). This classification is on one hand based on the length and composition of the fatty acid tail and on the other hand on the number, type, and configuration of the amino acids in the oligopeptide (Raaijmakers et al., 2006). Although different, these CLiPs generally have the similar mechanism of antimicrobial activity, being the integration into plasma membranes causing destabilization, and leakage or influx of ions leading to a disrupted cell multiplication or even cell death (Malviya et al., 2020). However, the exact mechanism is unknown for most CLiPs due to the constant discovery of new *Pseudomonas* strains, new compounds, and new groups of CLiPs (Dimkić et al., 2022). CLiPs produced by *Pseudomonas* spp. have also been proven to play a role in inducing systemicc resistance in plants (Ma et al., 2016; Tran et al., 2007). CLiPs play a role in biofilm formation and cell surface hydrophobicity, which is the reason a problem in the production of CLiPs has an effect on the biofilm structure and causes a loss in antifungal activity (Raaijmakers et al., 2010).

Cyclic lipopeptides produced by *Bacillus* spp. mostly belong to the surfactin, iturin and fengycin families (Fira et al., 2018). The kurstakin family of compounds was also discovered to have antifungal properties (Béchet et al., 2012). The molecular structures of these compounds are shown in Figure 5. Surfactins have antiviral, antimicrobial, hemolytic and antitumor properties (Dimkić et al., 2022; Seydlová et al., 2011). They are also able to self-assemble and form micelles (Seydlová et al., 2011). The antimicrobial properties of surfactins are based on their capability to cause membrane destabilization and leakage of cellular content (Dimkić et al., 2022; Seydlová et al., 2011). The compounds are able to penetrate the membrane bilayer due to hydrophobic interactions where they cause destabilization of the lipids and dehydration of the polar lipid heads (Dimkić et al., 2022; Seydlová et al., 2011). By forming micelles together with the phospholipids, the membrane permeability changes due to the formation of pores (Dimkić et al., 2022; Seydlová et al., 2011). The antagonistic activity of surfactins is dose-dependent whereby high doses can cause irreversible pore formation or even the complete disintegration of the membrane (Ongena & Jacques, 2008). The antimicrobial activity of fengycins is different than that of surfactins, but fengycins are also able to insert into the lipid bilayer where they can cause changes in its structure and permeability (Deleu et al., 2008). This causes fengycins to have not only antifungal and antibacterial activity, but also insecticidal activity (Dimkić et al., 2022). Iturins have antibiotic, antifungal and antitumor properties (Dimkić et al., 2022). The mechanism by which iturins exert antibiotic action also involves fungal membranes, but iturins cause the formation of ion-conducting pores (Falardeau et al., 2013). Pore formation is the result of the association of iturins with cations, resulting in disturbing osmotic regulation, cytoplasmatic leaking and cell death (Nakkeeran et al., 2019). There are also similarities with surfactins, since iturins also penetrate the cell wall, form aggregates with phospholipids and have an antagonistic activity that is dose-dependant (Falardeau et al., 2013). Iturins can also induce an immune system response in host plants (Aydi Ben Abdallah et al., 2019). It has been proposed that kurstakins are also pore forming compounds, although they have a lower amount of antimicrobial activity (Béchet et al., 2012).

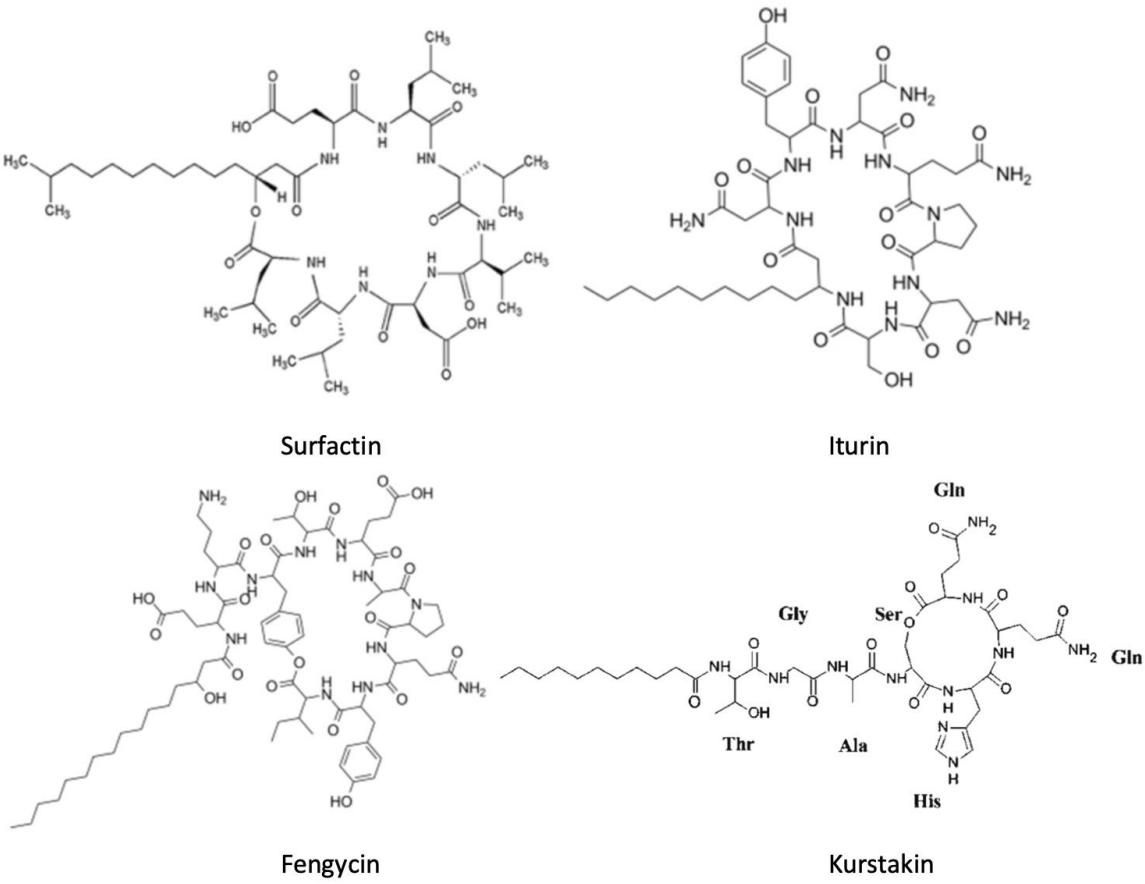


Figure 5. Molecular structures of surfactin, iturin, fengycin and kurstakin (Malviya et al., 2020; National Library of Medicine, n.d.).

Effectiveness of *Bacillus* spp. and *Pseudomonas* spp against *R. solani*

The effectiveness of combating *R. solani* by *Bacillus* spp. and *Pseudomonas* spp. and the compounds they produce has been the subject of a large number of studies. Both *Bacillus subtilis* and *Pseudomonas fluorescens* have been shown to be capable of reducing the growth of *R. solani in vitro* (El-Mougy & Abdel-Kader, 2008; Montealegre et al., 2003; S. Singh et al., 2021). Furthermore, El-Mougy & Abdel-Kader (2008) also showed that these bacteria were capable of decreasing the growth reduction of plants and symptoms of *R. solani in vivo* when applied as a bio-priming treatment on faba beans. *Bacillus subtilis* has further been proven to be an effective control agent in soils naturally infested with *R. solani*, significantly decreasing symptoms and increasing survival rate when applied on field sown with faba bean (Abou-Zeid et al., 2003). Similarly, *Pseudomonas fluorescens* has also found to be effective on faba bean production in soils naturally infested with *R. solani*, reducing disease incidence and improving growth and yield of the crop (Abd-El-Khair et al., 2021). Akladious et al. (2019) tested the effectiveness of biosurfactants produced by *Bacillus licheniformis* and concluded that disease incidence of *R. solani* on faba bean could be reduced when applied. Abdel-Monaim (2013) showed that *Bacillus megaterium* is able to reduce *R. solani* growth *in vitro* as well as reducing symptoms of *R. solani* on faba bean. Results also showed that fresh and dry weights of the plants increased when the biocontrol agent was applied, both in healthy and infected plants.

It has been shown that *Pseudomonas* strains RHF3.3-3 and UPB0736 were better in controlling *R. solani in vitro* than *Pseudomonas* strain COR33 (Ferrarini et al., 2022; Marahatta, 2021). This is possibly

due to the differences in CLiP production of these strains, with RHF3.3-3 producing CLiPs thanamycin, peptin19:5 and asplenin, UPB0736 producing syringotoxin, fuscopeptin A and B and asplenin while COR33 is only able to produce asplenin (Ferrarini et al., 2022; Marahatta, 2021; Oni et al., 2019, 2020; Patel et al., 2012). D'aes et al. (2011) found that *Pseudomonas sessilinigenes* CMR12a could significantly reduce root rot of *R. solani* infected Phaseolus beans, but mutants of the strain that lost the ability to produce phenazines or CLiPs were significantly worse in controlling the disease (Girard et al., 2021). Furthermore, Ma et al. (2016) showed that the production of either phenazines or CLiPs, either sessilins or orfamides, was necessary to induce systemic resistance to *R. solani* in Phaseolus bean. In another study conducted on *Pseudomonas sessilinigenes* CMR12a it was concluded that, depending on the soil composition, either phenazines or the CLiP sessilin or both were required for effective biocontrol of *R. solani* on Phaseolus bean (Hua & Höfte, 2015). Mnif et al. (2016) noted that iturin, fengycin and surfactin produced by *B. subtilis* strain SPB_1 showed significant antifungal activity against *R. solani in vitro*. Yu et al. (2002) purified three antifungal compounds from *Bacillus amyloliquefaciens* strain B94 and identified them as belonging to the iturin family. These compounds could inhibit the growth of *R. solani in vitro*, possibly playing a major role in the biocontrol activity of *B. amyloliquefaciens* together with the production of multiple enzymes by the bacteria. A different strain of *B. amyloliquefaciens*, being strain QST713, is currently the only biological control agent listed on Fytoweb (2023) that can be used to combat *R. solani* in Belgian agriculture.

3) Aim of the thesis

Seeing as we live in a world with a growing food and feed demand, it is important that we find methods of mitigating production losses of crops due to diseases. As explained earlier, *Rhizoctonia solani* can cause damage to a multitude of crops including faba bean. Cultural control methods and resistance breeding seem to not be very effective in controlling the disease. On the other hand, chemical control is associated with negative externalities like impact on the environment and pathogen resistance. That leaves biological control as a possible sustainable method of controlling *R. solani*. Fortunately, promising results using *Pseudomonas* and *Bacillus* species and the cyclic lipopeptides (CLiPs) they produce have already been found. This thesis will further examine the viability of these bacteria and CLiPs as a biocontrol agent against *R. solani* infections on faba bean.

The lab of phytopathology at the faculty of bioscience engineering at Ghent University possesses many *R. solani* isolates from Sweden belonging to different anastomosis groups (AGs), many of which belong to AG-5. Phylogenetic studies of these isolates based on the ribosomal DNA internal transcribed spacers (rDNA-ITS) gene region have been conducted at the lab. Their results suggest that AG-5 can be divided into two new subgroups, being AG-5-1 and AG-5-2 (Marcou, unpublished).

With this information in mind, the objectives of this thesis are outlined as the following:

- Further exploring the subdivision of AG-5 into two subgroups and relatedness of different AGs by means of studying hyphal anastomosis reactions.
- Determining the pathogenicity and aggressiveness of different *R. solani* isolates and AGs on faba bean.
- Evaluating the *in vitro* and *in vivo* biocontrol activity of different bacterial strains belonging to the genus *Pseudomonas* or *Bacillus* against different *R. solani* isolates and AGs.
- Evaluating the *in vitro* and *in vivo* effectiveness of the active substance fludioxonil in controlling different *R. solani* isolates and AGs.
- Evaluating the *in vitro* biocontrol activity of the different CLiPs produced by *Bacillus velezensis* strain GA1 (specifically surfactin, iturin and fengycin) against different *R. solani* isolates and AGs.

4) Methodology

4.1) Microorganisms

4.1.1) Used microorganisms

Several different isolates of *R. solani* and strains of *Bacillus* and *Pseudomonas* were used during this study. The bacterial strains are listed in Table 4 and the isolates of *R. solani* are listed in Table 5.

Table 4. List of the used bacterial strains during this thesis.

Strain	Species	CLiPs produced	Origin	Reference
RHF3.3-3	*Pseudomonas asplenii* G1	Thanamycin, Peptin19:5 Asplenin	Rice roots, Vietnam	Lam Bach, 2021
UPB0736	*Pseudomonas fuscovaginae*	Fuscopeptin A, Fuscopeptin B, Syringotoxin, Asplenin	Rice with sheath rot, Madagascar	Ferrarini et al., 2022; Patel et al., 2012
COR58	*Pseudomonas* U2 (sub)group	N4	Cocoyam roots, Cameroon	Oni et al., 2019, 2020
GA1	*Bacillus velezensis*	Iturin, Surfactin, Fengycin	Strawberry, Italy	Andric et al., 2021; Touré et al., 2004
GA1ΔsrfaA-ituA	*Bacillus velezensis*	Fengycin	GA1 mutant	Andric et al., 2021
GA1ΔsrfaA-fenA	*Bacillus velezensis*	Iturin	GA1 mutant	Andric et al., 2021
GA1ΔfenA-ituA	*Bacillus velezensis*	Surfactin	GA1 mutant	Andric et al., 2021

Table 5. List of the used *Rhizoctonia solani* isolates during this thesis.

Isolate*	Host plant	Year of isolation	Origin	Symptoms	Anastomosis group (AG)	Reference
RhPeVa-40 (40)	Pea	2017	Västergotland, Sweden	Brown epicotyl	AG-2-1	Unpublished
RhCaES-61 (61)	Carrot	2018	Eastern Scania, Sweden	Brown net of mycelium on leaf stems	AG-3	Marcou et al., 2021
RhFbGo-175 (175)	Faba bean	2021	Gotland, Sweden	Dark roots and epicotyl	AG-4 HGII	Unpublished
RhCaES-62 (62)	Carrot	2018	Eastern Scania, Sweden	Brown wilted stem bases/leaves	AG-5-1	Marcou et al., 2021
RhPeKa-139 (139)	Pea	2019	Kalmar, Sweden	Brown epicotyl	AG-5-1	Unpublished
GM-10	Soybean	/	Japan	/	AG-5-1	Schneider et al., 1997
RhSbES-15 (15)	Sugar beet	2016	Eastern Scania, Sweden	Damping-off	AG-5-2	Unpublished
RhPeVG-58 (58)	Pea	2017	Västergotland, Sweden	Brown epicotyl	AG-5-2	Unpublished
RhFbGo-176 (176)	Faba bean	2021	Gotland, Sweden	Dark roots and epicotyl	AG-5-2	Unpublished
RhSpES-16 (16)	Spinach	2016	Eastern Scania, Sweden	Damping-off	AG-11	Unpublished
RhCaES-20 (20)	Carrot	2015	Eastern Scania, Sweden	Greyish-white felt-like mycelium	AG-11	Marcou et al., 2021
RhSbWS-101 (101)	Sugar beet	2020	Western Scania, Sweden	Damping-off	AG-11	Unpublished

* Abbreviation is given between brackets. This will further be used to denote the isolates.

4.1.2) Growth media

The composition of the growth media used during this thesis are given in Table 6. Potato dextrose agar (PDA) was used to grow *R. solani* isolates. Kings' B (KB) agar and broth were used for the growth of *Pseudomonas* strains while Luria-Bertani (LB) agar and broth were used for *Bacillus* strains. For the anastomosis microscopy test, water agar was needed and 1/5 PDA was required for the *in vitro* antagonism experiments.

Table 6. Composition of the different growth media used.

Growth medium	Ingredients	Quantity
Potato dextrose agar (PDA)	Distilled water	1 L
	PDA powder	39 g
1/5 PDA	Distilled water	1 L
	Agar	15 g
	1/5 Potato dextrose broth	4.8 g
Kings' B (KB) agar	Distilled water	1 L
	Proteose pepton nr. 3 (Difco)	20 g
	K_2HPO_4	1.5 g
	$MgSO_4$	1.5 g
	Agar	15 g
	Glycerol	10 mL
Luria-Bertani (LB) agar	Distilled water	1 L
	Tryptone	10 g
	Yeast extract	5 g
	NaCl	10 g
	Agar	15 g
KB broth	Distilled water	1 L
	Proteose pepton nr. 3 (Difco)	20 g
	K_2HPO_4	1.5 g
	$MgSO_4$	1.5 g
	Glycerol	10 mL
LB broth	Distilled water	1 L
	Tryptone	10 g
	Yeast extract	5 g
	NaCl	10 g
Water agar	Distilled water	1 L
	Plant and fungi agar	15 g

4.1.3) Storage and growth of microorganisms

Rhizoctonia solani isolates, *Bacillus* and *Pseudomonas* strains were all stored in cryo-vials at -80 °C. For each *R. solani* isolate, 3 mycelial plugs of a 3-day old culture were stored in a mixture of 750 µL skimmed milk and 750 µL 40% sterile glycerol. For the storage of bacteria, 500 µL bacterial suspension grown overnight on liquid medium, being KB- or LB broth for *Pseudomonas*- or *Bacillus* strains respectively, was mixed with 500 µL sterile 40% glycerol.

When using *R. solani* isolates for an experiment, these were taken out of the cryostorage and placed on PDA medium about a week beforehand. After incubating at 28 °C for 3 to 4 days, mycelial plugs of the cultures were taken and placed on new PDA medium to again be incubated at 28 °C for another 3 to 4 days, until the cultures are used in an experiment.

When bacteria were used for the plant trials, 3 days before sowing the bacteria would be taken out of the cryostorage and placed on KB- or LB agar (for RHF3.3-3 or GA1 respectively) to incubate at 28 °C. One day later the bacteria were crisscrossed on new LB- or KB agar, meaning that the bacteria were spread out evenly over an entire plate with the goal of growing as many bacteria as possible. These plates were then incubated for another 2 days before the cultures were used.

Bacteria used for the *in vitro* antagonism tests were also taken out of the cryostorage 3 days before the experimental setup and placed on KB- or LB agar (for *Pseudomonas- or Bacillus* strains

respectively) to incubate at 28 °C. One day later the bacteria were transferred to new KB- or LB agar and again incubated at 28 °C. One day before the experimental setup, the bacteria were transferred to KB- or LB broth and grown in a shaker at 28 °C.

4.2) Anastomosis microscopy test

For the first experiment, the relatedness of different *R. solani* isolates used during this thesis was researched by determining the degree of anastomosis between the different isolates. The isolates used for this experiment are listed in Table 7. The goal was mainly to further explore the subdivision of AG-5 into 2 subgroups. Isolate GM-10 is used as a tester isolate for AG-5-1. An isolate of the AG-4, which is unrelated to AG-5, was also included in the experiment together with 2 isolates belonging to the more closely related AG-11.

Table 7. *Rhizoctonia solani* isolates used during the anastomosis microscopy test.

Isolate	175	62	139	GM-10	15	58	176	16	101
Anastomosis group (AG)	4 HGII	5-1	5-1	5-1	5-2	5-2	5-2	11	11

To test anastomosis, 2 mycelial plugs from 4-day old cultures, measuring 3 mm in diameter were placed on 300 µL water agar, 3 cm apart from one another on a sterile microscopic slide. The microscopic slides were then placed on 2 sterile toothpicks in a petri dish containing sterile filter paper that was made wet using sterile distilled water. This was done for each combination of isolates, each time preparing 3 replicas. An illustration of the setup is shown in Figure 6. These petri dishes were then placed in a 28 °C incubator until the hyphae have grown to the point where there is contact between the 2 isolates. Depending on the isolates, this took 1 to 4 days. When contact was observed, the hyphae are evaluated under a light microscope (Olympus BX51) to detect anastomosis. The anastomosis reactions were classified according to the terminology described by Carling et al. (1988) listed in Table 1 (section 2.2.2).

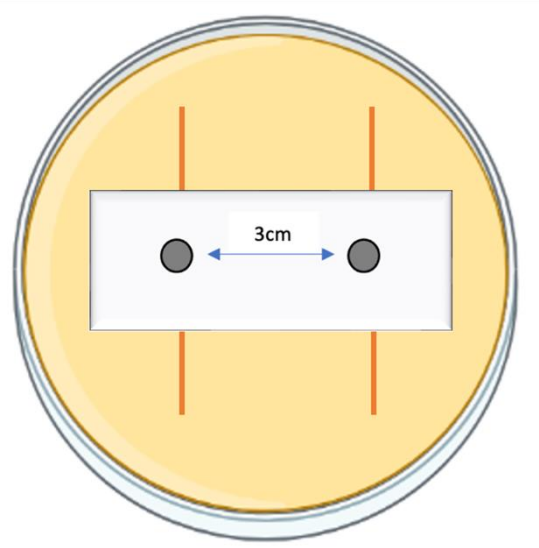


Figure 6. Setup of a microscopic plate in a petri dish used in the anastomosis microscopy test.

4.3) Plant trials

4.3.1) Plants and planting material

During plant trials, faba beans (*Vicia faba* L.) from the company Aveve (2022) were used. These are sold under the commercial name Scorpio. The substrate in which the seeds are sown is made up on weight-based equal parts potting soil (universal type 2 structural, Snebbout N.V., Belgium; 15% organic matter, pH 5-6.5 and E.C. of 350 μS/cm)) and river sand. Once sown, the plants are kept in growth chambers that have a temperature of 24°C, a day and night regime consisting of 16 hours of light and 8 hours of darkness and a relative humidity of 60%.

4.3.2) Inoculum preparation of *Rhizoctonia solani*

In each plant trial, it was necessary to infect the faba bean plants with different isolates of *R. solani*. In order to do this, inoculum of the different isolates was needed. For this, wheat kernels covered in mycelium were used. First, the kernels were soaked in water overnight after which 100 mL Erlenmeyer flasks were filled with about 60 g of kernels. These flasks were then covered with a cotton wool prop and aluminum foil and autoclaved twice on consecutive days. Per isolate, 5 mycelial plugs were then added to a flask. Finally, the inoculum flasks were incubated at 28°C for 9 to 11 days while being shaken every 3 days to prevent coagulation of the kernels.

4.3.3) Determination of field capacity

From the second plant test and onwards, plants were given water in order for the substrate to be above 75-80% field capacity to avoid drought-stress. For this to be carried out, the field capacity of the substrate used in these tests needed to be determined. This was achieved by filling 4 plant pots with 140 g substrate and giving these an excessive amount of water. The plant pots where then placed in larger containers that could catch the leaking water. Foil was placed over the plant pots in order to create a closed environment. The substrate was determined to be at field capacity when the soil was sticky but not tangibly wet. The soil moisture content was measured 4 times per pot using a moisture meter connected to a sensor (HH2 and SM300, AT Delta-T Devices, Cambridge, England) on 4 consecutive days and the weight of the pots was measured on the last 2 days of the experiment. When watering the plants during plant trials, the soil moisture content and the weight of the plants was measured. The plants could then be given water based on the measurements from the last day when the substrates were at field capacity. The plants were never watered directly but always by pouring the water in a tray in which the plant pots were placed.

4.3.4) Determination of pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates on *Vicia faba* L. (plant trial 1)

In the first plant trial, the pathogenicity and aggressiveness on faba bean of different *R. solani* isolates, listed in Table 8, was tested. Pathogenicity meaning the capability of the isolates to infect faba bean plants and aggressiveness meaning the disease severity this infection causes. Four-day old cultures of *R. solani* were used to prepare the inoculum.

Table 8. *Rhizoctonia solani* isolates used during plant trial 1.

Isolate	175	62	139	15	58	176	16	20	101
Anastomosis group (AG)	4 HGII	5-1	5-1	5-2	5-2	5-2	11	11	11

Before sowing, the faba bean seeds were sterilized and pregerminated. This process began by first soaking the seeds in a 1% NaClO solution for 5 minutes. The seeds are then washed 3 times with sterile distilled water and left to dry on sterile filter paper. When dry, the seeds are placed on a sterile piece of cotton wool in a petri dish. The seeds are placed in the dishes in batches of 15 and each dish is given 5 mL of distilled water. When this is done, they are left in a 28 °C incubator for 3 days after which the seeds are sown. Trays are filled up with 700 g of the aforementioned substrate. Per tray, 10 seeds are sown in 2 rows of 5 seeds. Holes had been poked in the bottom of each plant tray and these plant trays are then placed in into watertight trays. By doing this, watering can be done by pouring water in the second tray instead of directly on the plants. The plant trays are covered with plastic foil and given 150 mL of water. After 5 days, the foil is removed and the seeds are again given 150 mL of water.

Eight days after sowing and 9 days after the inoculum was prepared, the plants were inoculated with the different *R. solani* isolates. Due to suboptimal sprouting, plant trays were selected in order for each treatment with *R. solani* isolates to include 12 to 14 plants instead of the originally planned 20. The leftover plants were not inoculated and used as healthy control treatment. The plants were inoculated by digging a small trench in each tray between the 2 rows of plants and placing 40 infected wheat kernels in the trench. Wheat kernels that were sufficiently covered by mycelium were hand selected. After the inoculation, the plants were given 150 mL of water which also occurred the following 4 days. 200 mL of water was given 2 days before the evaluation and no water was given the day before the evaluation.

Seven days after inoculation, the plants were evaluated on disease severity. First, the plants were removed from the substrate and washed in order for the symptoms of *R. solani* the be clearly visible. The disease severity was estimated by using a 0 to 4 scoring system shown in Table 9 and Figure 7. Using this scoring system, the disease severity index (DSI) can be calculated using the following formula.

$$\text{DSI } (\%) = \frac{\Sigma(\text{Class value} * \text{Class frequency})}{\text{Total number of plants} * \text{Maximal class value}} * 100$$

Table 9. Disease scoring system used to evaluate the disease severity of *Rhizoctonia solani* during plant trials.

Score	***Rhizoctonia solani* symptoms**
0	Healthy plants, absence of symptoms
1	Black or brown-colored lesions, smaller than 1mm in diameter
2	Black or brown-colored lesions covering less than 75% of the stem/root surface
3	Black or brown-colored lesions covering more than 75% of the stem/root surface
4	Dead seedlings

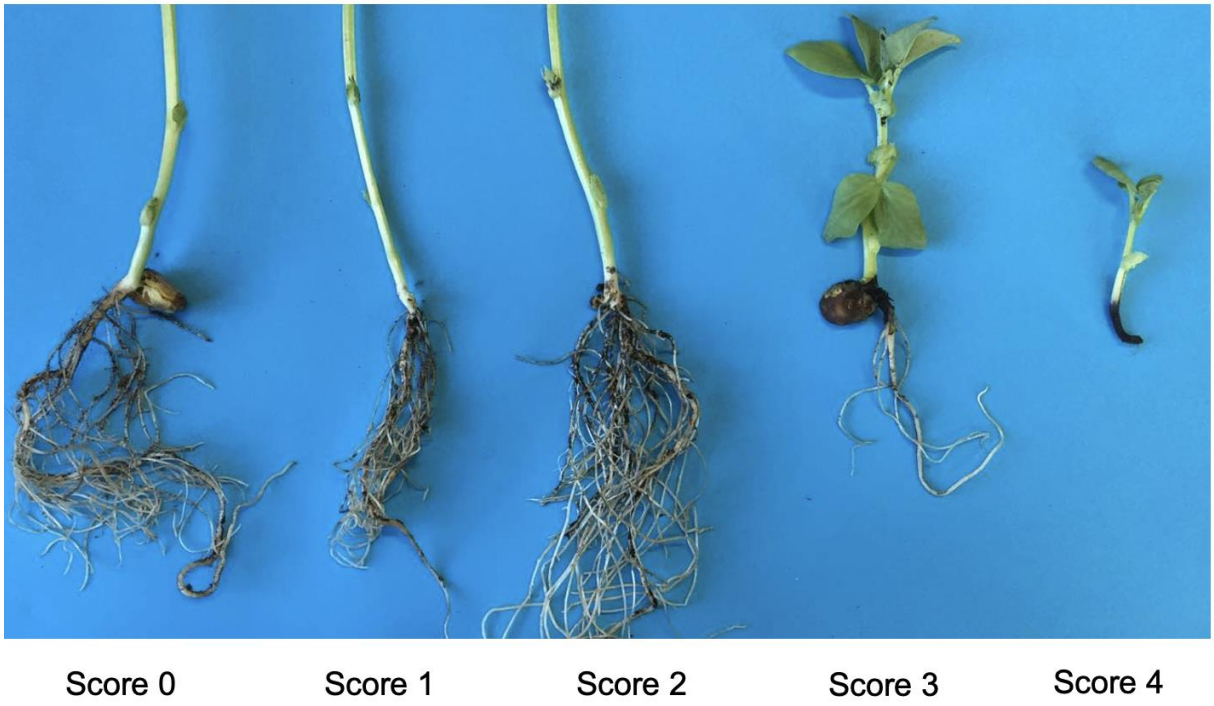


Figure 7. Visualization of the disease scale used to evaluate the disease severity of *Rhizoctonia solani* during plant trials.

4.3.5) Determination of pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates on *Vicia faba* L. (plant trial 2)

Because no definitive conclusion could be reached based on the first plant trial, a similar plant trial was conducted. The *R. solani* isolates used for this experiment were the same as for plant trial 1, with the addition of isolates 40 (AG-2-1) and 61 (AG-3). This time, 3-day old cultures were used to prepare the inoculum. There was only one difference in the sterilization and pregermination of the faba bean seeds compared to the first plant trial. Now 20 mL of water was added to the dishes, containing the seeds and cotton wool, prior to the 3-day incubation at 28 °C.

Instead of in trays, the seeds were sown individually in sterilized plant pots containing 140 g substrate. The plant pots were placed in trays and were now given water in order for the substrate to be at 75-80% field capacity, as explained in section 4.3.3. Table 10 contains the quantities of water given to each plant throughout the experiment. The plants were inoculated 7 days after sowing and 11 days after the preparation of the inoculum. This was carried out by digging a trench around each plant and placing 8 hand selected infected wheat kernels per plant in the trench. For each infected treatment and the healthy control treatment, 8 to 10 plants were used. Unfortunately, the inoculum flasks of isolates 15 and 139 were contaminated and could not be used for this experiment. The plants were evaluated 10 days after inoculation, in the same manner as in the previous experiment. In addition, the length (from the bottom of the roots to the top of the plant) and weight of the plants were also measured.

Table 10. Quantities of water given to each plant during plant trial 2.

Days after sowing	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Quantity of water given per pot (mL)	50	30	0	30	0	50	0	0	0	50	0	30-50*	0	50	0	0	50

* Amount of water depended on the soil moisture content of the individual plant pots.

4.3.6) Testing of disease control capability of *Bacillus velezensis* strain GA1 and fludioxonil against *Rhizoctonia solani* inoculated *Vicia faba* L. (plant trial 3)

In this experiment, the disease control of GA1 and fludioxonil were tested *in vivo* against different strains of *R. solani* infecting faba bean. First, inoculum was prepared using 4-day old cultures of the *R. solani* isolates listed in Table 11.

Table 11. *Rhizoctonia solani* isolates used during plant trial 3 and *in vitro* antagonism tests 1 and 2.

Isolate	61	175	139	58	16
Anastomosis group (AG)	3	4 HGII	5-1	5-2	11

On the day of sowing, bacterial solutions were prepared and faba beans seeds were coated in fludioxonil. The bacterial solutions were made by scraping of the bacteria from 2-day old, crisscrossed plates of GA1 and pouring these in 100 mL Erlenmeyer flasks. The flasks were then filled up to 100 mL with tap water. Using a 96 well plate and a spectrophotometer, the optical density at 620nm (OD_{620}) of the bacterial solutions and tap water as control were measured. By first subtracting the $OD_{620,\ control}$ value from $OD_{620,\ bacteria}$ and then multiplying this value by 822.93, a value for the number of Colony Forming Units (CFUs) * 10^6/mL in the bacterial solution was found. This value was then adjusted to attain a quantity of 10^7 CFUs/g when adding the bacterial solution to the substrate. Faba bean seeds were coated using a sowing seed coater (Wintersteiger, Germany) that was first sterilized using 70% ethanol. Pure Celest, containing the active substance fludioxonil, was ½ diluted and 600 mL/100 kg seeds was added drop by drop to the atomizer of the coater. This is 1.5 times the amount recommended by Fytoweb (2023) in order to assure a proper coating of the fungicide. The seeds were drop by drop treated in the drum of the coater and dried after the fungicide was applied. This resulted in seeds on average coated with 32 µg of fludioxonil. This is less than was expected, probably due to the large size of the seeds which causes loss of fungicide in the coater. The process did result in an amount of fludioxonil coating that is close to the amount (Fytoweb, 2023) recommends, being around 35 µg per seed.

Since pregermination did not seem to be very effective, this time only surface-sterilization was carried out. The seeds were soaked in a 1% NaClO solution for 5 min, washed 3 times with sterile distilled water and left to dry on sterile filter paper before sowing. Sowing occurred in 3 batches. The first was a control batch, where regular seeds were individually sown in 140 g of substrate to which 20 mL of water had been added. In the second batch, the same amount of substrate and water were used but this time, faba bean seeds coated in fludioxonil were sown. In the final batch, regular seeds were first soaked in the bacterial solution. Each time, 15 seeds were soaked in 10 mL of the solution in a sterile petri dish. After soaking, the seeds were sown in 140 g of substrate to which 20 mL of bacterial solution had been added. The plants were again given water in order for the substrate to reach 75-80% field capacity. Table 12 shows the amount of water given in this experiment. Seven days after sowing and

10 days after preparing the inoculum, the plants were inoculated using the same method as in plant trial 2. For each isolate and the healthy control treatment, 10 pots from each batch were used, but only 5-7 plants per treatment had sprouted from those pots at the moment of inoculation. This number did increase by the time the plants were evaluated, which was 7 days later. The evaluation was again the same as in plant trial 2, measuring the disease severity, length and weight of the plants.

Table 12. Quantities of water given to each plant during plant trial 3.

Days after sowing	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Quantity of water given per pot (mL)	50	0	30-50*	0	30-50*	0	40-50*	0	0-30*	0	50	0	0	50

* Amount of water depended on the soil moisture content of the individual plant pots.

4.3.7) Testing of disease control capability of *Pseudomonas asplenii* G1 strain RHF3.3-3 and fludioxonil against *Rhizoctonia solani* inoculated *Vicia faba* L. (plant trial 4)

This experiment had the same goal and setup as plant trial 3, with the difference being that now RHF3.3-3 was evaluated for its biocontrol activity instead of GA1. Inoculum was prepared using 3-day old cultures of the same *R. solani* isolates used in plant trial 3. Unfortunately, isolate 16 was not able to grow and could not be used in this experiment. The bacterial solution of RHF3.3-3 was prepared in the same manner as explained in plant trial 3 and the same fludioxonil coated faba beans were used. As in plant trial 3, the seeds were only sterilized and not pregerminated, after which they were again sown in 3 batches. Water was also given to the plants in the same manner as in the previous 2 plant trials. The amount of water given to the plant is listed in Table 13. Seven days after sowing and 9 days after the inoculum had been prepared, the plants were inoculated using the same method as in plant trial 2 and 3. For each isolate and the healthy control treatment, 10 pots from each batch were used. There was however a discrepancy in germination between the different batches. In the fludioxonil batch, enough plants had sprouted to use 8-9 plants per treatment and in the control batch 7-8 plants per treatment could be used. However, in the RHF3.3-3 batch only 4-5 plants could be used per treatment due to low amount of germination. The evaluation was carried out 7 days after the inoculation and again consisted of measuring the disease severity, weight and length of the plants.

Table 13. Quantities of water given to each plant during plant trial 4.

Days after sowing	0	1	2	3	4	5	6	7	8	9	10	11	12
Quantity of water given per pot (mL)	50	0	30-50*	0	30	0	30-50*	0	30-40*	0	40-50*	0	40-50*

* Amount of water depended on the soil moisture content of the individual plant pots.

4.4) *In vitro* tests

4.4.1) *In vitro* antagonism of *Pseudomonas* spp. and *Bacillus* spp. against *Rhizoctonia solani* (*in vitro* antagonism test 1)

In this experiment, the *in vitro* antagonism of 4 different bacterial strains were tested against different *R. solani* isolates. The strains of bacteria used are COR58, RHF3.3-3, UPB0736 and GA1. Bacterial solutions were prepared by first measuring the OD_{620} value of the cultured bacteria in KB- or LB broth using a microplate reader (Infinite M Plex, Tecan). The solutions were then diluted with KB- or LB broth

to an OD_{620} value of 1. Four-day old cultures of the *R. solani* isolates listed in Table 11 (section 4.3.6) were used.

The experiment was carried out by making dual cultures, being the coculturing of 2 organisms on the same medium. In the middle of a 1/5 PDA plate, a mycelial plug of the used *R. solani* isolate was placed. Two droplets of 10 µL of the used bacterial solution was placed 2 cm from the central plug. For each combination of *R. solani* isolate and bacterial strain, 8 replicates were made of which 4 were incubated at 28 °C and 4 were incubated at 20 °C. The setup of a plate is shown in Figure 8. In the control treatment, only the *R. solani* plug was placed on the plate. Three days and 7 days later, pictures from the plates were taken. The surface area of the mycelium was calculated through the use of imaging software ImageJ. These surface areas were used to calculate the Percentage Area Inhibition (PAI) values using following formula.

$$\text{PAI } (\%) = \frac{\text{Colony Area of Control} - \text{Colony Area of Treatment}}{\text{Colony Area of Control}} * 100$$

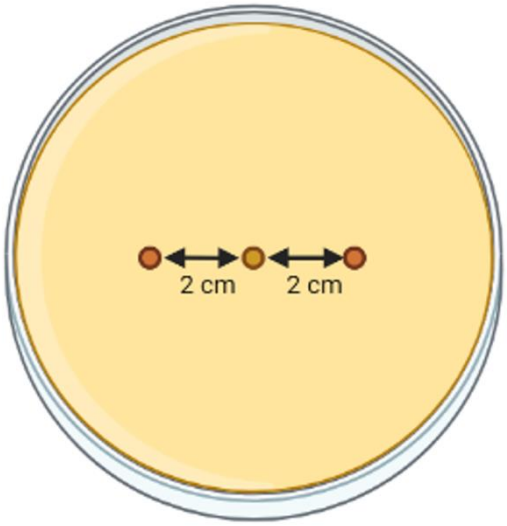


Figure 8. Setup of a 1/5 potato dextrose agar plate used in the *in vitro* antagonism experiments (Credits: Shirley Marcou, Ghent University).

4.4.2) *In vitro* antagonism of *Bacillus velezensis* strain GA1 mutants against *Rhizoctonia solani* (*in vitro* antagonism test 2)

In this experiment, the *in vitro* antagonism of GA1 and GA1 mutants that only produce one kind of cyclic lipopeptide (CLiP) (specifically GA1ΔsrfaA-ituA, GA1ΔsrfaA-fenA and GA1ΔfenA-ituA) were tested against different *R. solani* isolates. Table 4 (section 4.1.1) lists the CLiPs that these strains are capable of producing. The bacterial solutions were prepared in the same way as explained in *in vitro* antagonism test 1. Three-day old cultures of the *R. solani* isolates listed in Table 11 (section 4.3.6) were used. The rest of the experiment follows the exact same methodology used during *in vitro* antagonism test 1.

4.4.3) *In vitro* effectiveness of fludioxonil against *Rhizoctonia solani* (*in vitro* fludioxonil test)

In the final experiment, the *in vitro* effectiveness of fludioxonil was tested against different isolates of *R. solani*, listed in Table 14.

Table 14. Isolates of *Rhizoctonia solani* used during *in vitro* fludioxonil test.

Isolate	61	175	62	139	15	58	176	16	101
Anastomosis group (AG)	3	4 HGII	5-1	5-1	5-2	5-2	5-2	11	11

For this experiment, PDA plates with different concentrations of fludioxonil, using the commercial fungicide Pure Celest, were made. This was done by preparing a dilution series of a stock solution with a concentration of 25 000 µg/mL fludioxonil. Each time, 4 mL of a dilution was added to 996 mL of PDA medium. This was done by first mixing 39 g PDA powder with 900 mL distilled water and autoclaving this mixture along with regular distilled water. Then 4 mL of the used dilution was micropipetted into a 50 mL falcon after which the falcon was filled up to 50 mL with sterile distilled water and added to the PDA mixture. Finally, another 50 mL of sterile distilled water was added to the mixture to obtain the desired concentration of fludioxonil in the PDA medium. The different concentrations of fludioxonil used in the PDA medium of this experiment alongside the concentrations of the dilution series are given in Table 15.

Table 15. Different concentration of fludioxonil in the PDA medium used during the *in vitro* fludioxonil test along with the dilution factors of the stock solution (25 000 µg/mL fludioxonil and the concentrations of fludioxonil in the dilutions.

Dilution factor of the stock solution	**Concentration of fludioxonil in the dilution (µg/mL)**	**Concentration of fludioxonil in the PDA medium (µg/mL)**
/	0	0
1/10 000	2.5	0.01
1/4000	6.25	0.025
1/2000	12.5	0.05
1/1000	25	0.1
1/200	125	0.5
1/100	250	1
1/10	2500	10

The experiment was set up by first drawing two perpendicular lines that meet in the center on the bottom of each plate. A mycelial plug of a 4-day old *R. solani* culture was then placed in the center of the plates. Four replicates were made of each combination of fludioxonil concentration and *R. solani* isolate. These plates were put in an incubator at 25 °C. Each of the following 3 days, the lines would be marked to where the mycelium had grown, as to obtain 2 “diameters” of the mycelium. By averaging these diameters, a value is found that indicates the growth the isolate. The average diameter can then be used to calculate the Growth Inhibition (GI) of each combination of fludioxonil concentration and *R. solani* isolate using the following formula.

$$\text{GI}\ (\%) = \frac{\text{Diameter of Control} - \text{Diameter of Treatment}}{\text{Diameter of Control}} * 100$$

Using the GIs calculated on the final day of the experiment, a four-parameter log-logistic estimation of the half maximal effective concentration (EC_{50}) was made of fludioxonil for each *R. solani* isolate used. The EC_{50} value represents the concentration of, in this case, fludioxonil that is needed to obtain 50% GI. Because four replicates were prepared for each combination of fludioxonil concentration and *R. solani* isolate, four estimations of the EC_{50} value could be made. This was done using the drc package (Version 3.0-1) in Rstudio (Version 2023.03.0+386).

4.5) Statistical data analysis

The statistical data analysis was conducted in Rstudio (Version 2023.03.0+386). Analyses were carried out for each experiment except the anastomosis microscopy test, due to the low number of replicates in this experiment. First, a Kolmogorov-Smirnov test was used to assess if the data were normally distributed. This was not the case for the disease-score data from the plant trials. Here, a non-parametric Kruskal-Wallis test would be carried out to compare multiple treatments followed by a post-hoc Dunn's test to compare two individual treatments. The other data were normally distributed. Here, a Levene test was used to assess if the data were homo- or heteroscedastic. For homoscedastic data, the one-way ANOVA test and post-hoc Tukey test were used to compare the means of different treatments. For heteroscedastic data, a Brown-Forsythe test and post-hoc Scheffe test were applied to compare the means. A significance level of 5% was used for each test, except the Levene test, where a significance level of 1% was used. As most normally distributed data were found to be homoscedastic, it is only indicated when data were heteroscedastically distributed.

5) Results

This thesis entailed several different experiments. The first was the anastomosis microscopy test, carried out in order to have a better understanding of the relatedness of the different *R. solani* isolates and AGs that were used throughout the thesis. Secondly, plant trials were carried out with the goal of finding the most aggressive isolates of different AGs on faba beans. These isolates were then used to test different bacterial strains for their biocontrol potential. The first step in this process was an *in vitro* antagonism test where growth inhibition of *R. solani* caused by different *Bacillus* and *Pseudomonas* strains was evaluated. After this initial screening, the best performing bacteria were selected to further evaluate their biocontrol activity *in vivo* and compare this to a more traditional chemical control method using fludioxonil. After these plant trials, an additional *in vitro* evaluation of the effectiveness of fludioxonil was carried out. To round out the experiments, mutants of *Bacillus velezensis* strain GA1 were used for a second *in vitro* antagonism test with the goal of determining which CLiP(s) produced by GA1 is/are responsible for its antifungal activity.

5.1) Anastomosis microscopy test

The relatedness of different isolates of *R. solani* was tested in the anastomosis microscopy test. Phylogenetic studies of the rDNA-ITS gene region have suggested that AG-5 can be divided into two new subgroups, namely AG-5-1 and AG-5-2 (Marcou, unpublished). The main goal of this experiment was to further explore this subdivision of AG-5 using a more traditional anastomosis test. This was done by observing the reactions between isolates belonging to AG-4 HGII, AG-5-1, AG-5-2 and AG-11. Isolate GM-10 has been determined to belong to AG-5-1 based on analysis of the rDNA-ITS gene region, which is why it was included as a tester isolate for this subgroup. Isolate 175 of AG-4 HGII is thought to be unrelated to AG-5 and is used as a type of control isolate. Isolates belonging to AG-11 were also tested as this AG is thought to be more closely related to AG-5. The types of anastomosis reactions observed, based on the C0-C3 classification system of Carling et al (1988) (Table 1, 2.2.2), are shown in Table 16. Unfortunately, for some replicates a classification could not be made. This was because the hyphae of the isolates had grown too much, making possible anastomosis reactions unclear.

Table 16. Types of anastomosis reactions based on the classification of Carling et al. (1988) between different *Rhizoctonia solani* isolates belonging to AG-4 HGII, AG-5 and AG-11, observed during the anastomosis microscopy test. GM-10 was used as a tester isolate for AG-5-1. Green indicate C3 reactions, orange indicate C2 reactions, yellow indicate C1 reactions, red indicate C0 reactions and black indicate that no reaction could be observed.

Anastomosis Group (AG)		AG-4 HGII	AG-5-1			AG-5-2			AG-11	
	Isolate	175	62	139	GM-10	15	58	176	16	101
AG-4 HGII	175	C3 C3 C3								
AG-5-1	62	C0 C0 C0	C3 C3 C3							
	139	C0 C0 C0	C3 C3 C3	C3 C3 C3						
	GM-10	C0 C0 C0	C3 C3 C3	C3 C3 C3	C3 C3 black					
AG-5-2	15	C0 C0 C0	C2 C2 black	C2 C2 C2	C2 C2 C2	C3 C3 C3				
	58	C0 C0 C0	C2 C2 C2	C2 C2 C2	C2 C2 C2	C3 C3 C3	C3 C3 C3			
	176	C0 C0 C0	C2 C2 C2	C2 C2 C1	C2 C2 C2	C3 C3 C3	C3 C3 C3	C3 C3 C3		
AG-11	16	C0 C0 C0	C0 C0 C0	C0 C0 C0	C0 C0 C0	C0 C0 C0	C0 black C0	C0 C0 C0	C3 C3 black	
	101	C0 C0 C0	C1 C0 C0	C1 C0 black	C0 C0 C0	C0 C0 C0	C1 C1 C0	C0 C0 C0	C3 C3 C3	C3 C3 black

As expected, each time self-anastomosis was tested, C3 reactions were observed. Isolates belonging to AG-5-1, namely isolates 62 and 139 and the tester isolate GM-10, predictably also displayed C3 reactions amongst each other. This was also the case for isolates 15, 58 and 176 of AG-5-2 as well as isolates 16 and 101 of AG-11.

Isolate 175 of AG-4 HGII did not display any type of anastomosis reaction with any other isolate or AG. When isolates belonging to AG-5-1 were paired up with isolates belonging to AG-5-2, typically C2 reactions were observed. There was one exception when isolate 139 was paired up with isolate 176, where one C1 reaction was observed. Interestingly, C1 reactions also took place when isolate 101 of AG-11 was paired up with isolates belonging to AG-5. One C1 reaction took place when paired up with isolate 62 and one when paired up with isolate 139, both belonging to AG-5-1. Another two occurred

when isolate 101 was paired up with isolate 58 of AG-5-2. There were otherwise no anastomosis reactions observed between the isolates of AG-5 and AG-11.

5.2) Determination of pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates on *Vicia faba* L. (plant trial 1)

In plant trial 1, the pathogenicity of different *R. solani* isolates on faba bean was assessed by comparing *R. solani* treatments to an uninoculated control. By comparing the isolate treatments with each other, the differing levels of aggressiveness on faba bean could be analyzed. This analysis was carried out using the disease scoring system listed in Table 9 and shown in Figure 7 (section 4.3.4). These results are shown in Figure 9. The disease severity index (DSI) was also calculated for each treatment and is given in Table 17. As can be seen below, multiple diseased plants were observed in the uninoculated control, leading to a DSI greater than zero. For consistency, the uninoculated control will be called the healthy control from now on.

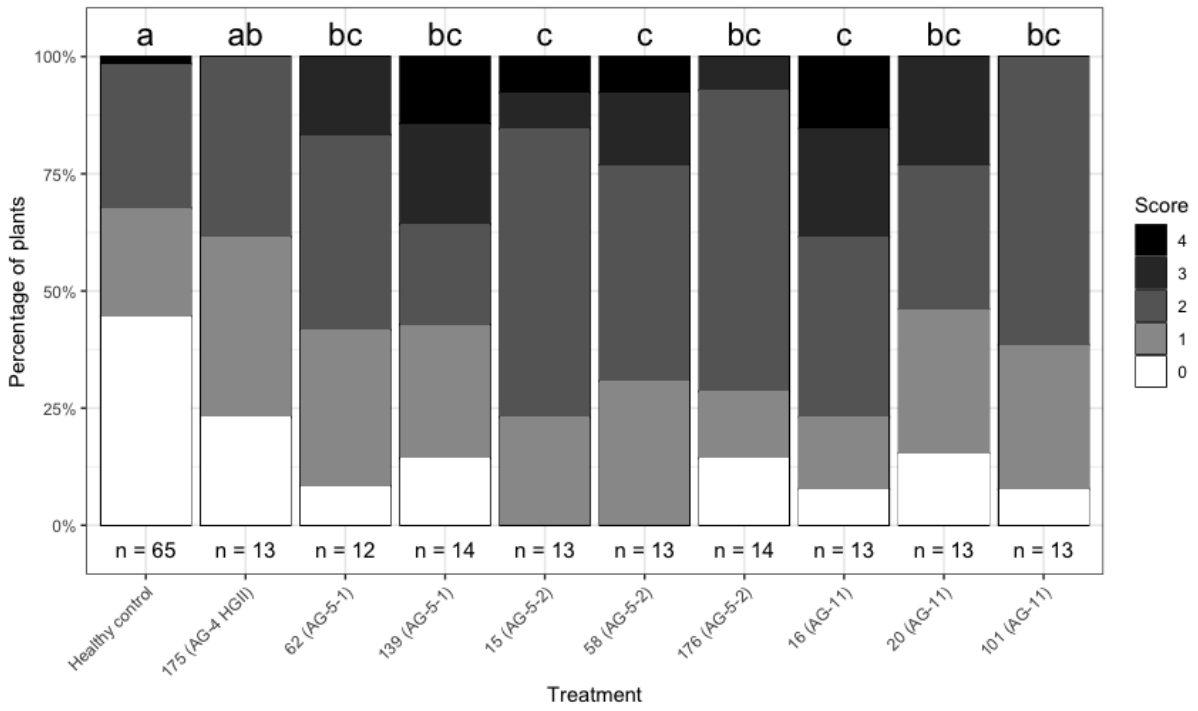


Figure 9. Observed disease severity of *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control on faba bean in plant trial 1, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate a statistically significant difference in disease severity between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

Of all the isolates, only plants treated with isolate 175 (AG-4 HGII) did not have a significantly higher disease severity than the healthy control. However, there was no significant difference between plants inoculated with isolate 175 and those that were inoculated with isolates 62 (AG-5-1), 139 (AG-5-1), 176 (AG-5-2), 20 (AG-11) and 101 (AG-11). Treatments with isolates 15 (AG-5-2), 58 (AG-5-2) and 16 (AG-11) did result in a significantly higher disease severity than the treatment with isolate 175. However, the disease severity of these plants was again not significantly different from plants treated with isolates 62, 139, 176, 20 and 101.

Table 17. The disease severity indices (DSI) of *Rhizoctonia solani* isolates and a healthy control on faba bean in plant trial 1. The average DSI per anastomosis group is also given.

Anastomosis group (AG)	Treatment	Disease Severity Index (DSI) (%)	Average DSI per AG (%)
/	Healthy control	22.69	/
AG-4 HGII	175	28.85	28.85
AG-5-1	62	41.67	44.94
	139	48.21	
AG-5-2	15	50.00	47.02
	58	50.00	
	176	41.07	
AG-11	16	55.77	44.87
	20	40.38	
	101	38.46	

Even though multiple diseased plants were observed, the healthy control still had the lowest DSI of all treatments. Of all the plants treated with *R. solani*, treatment with isolate 175 resulted in the lowest DSI. Of the isolates belonging to AG-5-1, treatment with isolate 139 resulted in the highest DSI and treatments with isolates 15 and 58 had the highest DSI of isolates belonging to AG-5-2. Plants inoculated with isolate 16 had the highest DSI of AG-11, which was also the highest DSI overall. Except for AG-4 HGII, of which only one isolate was tested, no substantial differences were observed in the average DSI of each AG.

5.3) Determination of pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates on *Vicia faba* L. (plant trial 2)

The pathogenicity and aggressiveness of different *R. solani* isolates on faba bean was again evaluated in plant trial 2. A different setup and some isolates differing from plant trial 1 were used. In this experiment, the height and weight of the plants were also measured and could be compared between treatments along with the disease score and DSI. The results of the disease scoring are given in Figure 10 and the DSIs of different treatments are given in Table 18. This time, no diseased plants were observed in the healthy control.

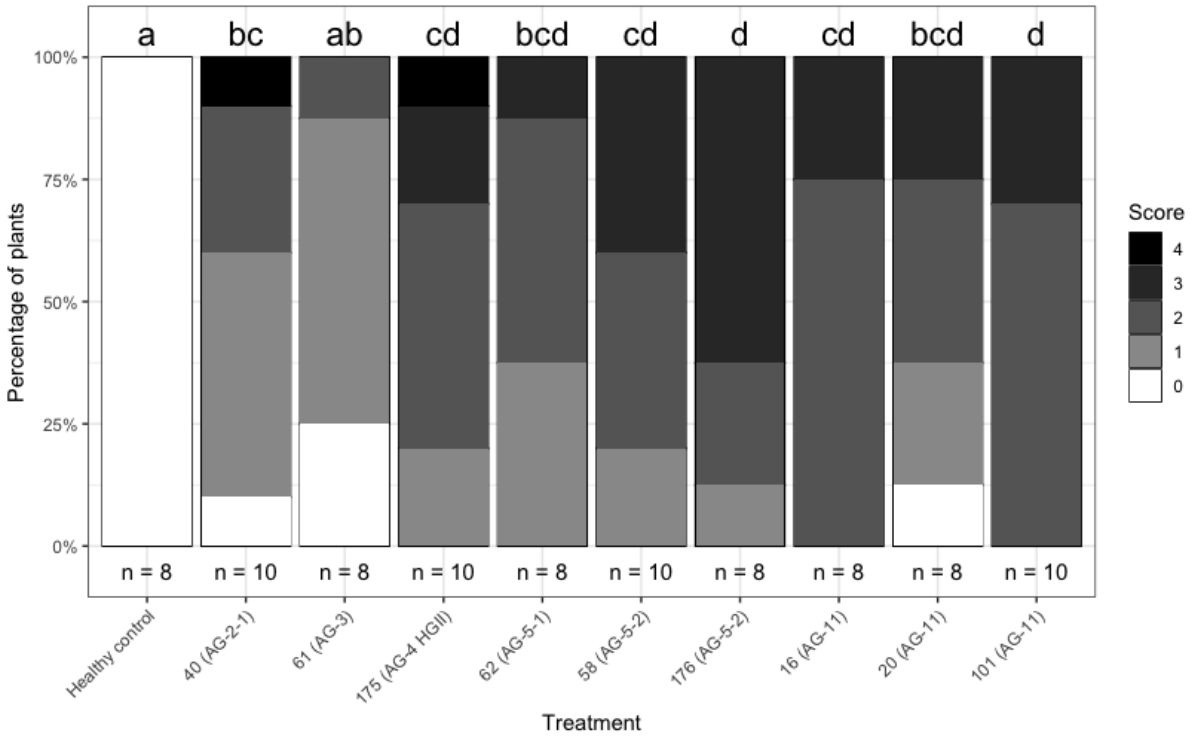


Figure 10. Observed disease severity of *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control on faba bean in plant trial 2, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate a statistically significant difference in disease severity between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

Again, only one treatment with an isolate did not result in a significantly higher disease severity than the healthy control, this time being isolate 61 (AG-3). Treatments with isolates 40 (AG-2-1), 62 (AG-5-1) and 20 (AG-11) resulted in a significantly higher disease severity than the healthy control, but not higher than the treatment with isolate 61. Plants treated with isolates 175 (AG-4 HGII), 58 (AG-5-2) and 16 (AG-11) did have a significantly higher disease severity than those treated with isolate 61, but not those treated with isolates 40, 62 and 20. Finally, plants inoculated with isolates 176 (AG-5-2) and 101 (AG-11) showed the highest level of disease severity, being significantly higher than those inoculated with isolate 40 or 61, but not those inoculated with isolates 175, 62, 58, 16 and 20.

Table 18. The disease severity indices (DSI) of *Rhizoctonia solani* isolates and a healthy control on faba bean in plant trial 2. The average DSI per anastomosis group is also given.

Anastomosis group (AG)	Treatment	Disease Severity Index (DSI) (%)	Average DSI per AG (%)
/	Healthy control	0.00	/
AG-2-1	40	37.50	37.50
AG-3	61	21.88	21.88
AG-4 HGII	175	55.00	55.00
AG-5-1	62	43.75	43.75
AG-5-2	58	55.00	57.19
	176	59.38	
AG-11	16	56.25	52.50
	20	43.75	
	101	57.50	

From all the plants treated with *R. solani*, those treated with isolate 61 resulted in the lowest DSI. From the treatments that showed a significantly higher disease severity than the healthy control, isolate 40 resulted in the lowest DSI and isolates 62 and 20 in the second lowest. All other treatments had a disease severity above 50%. In this plant trial, inoculation with isolate 175 resulted in the fourth highest DSI, tied with isolate 58. Treatment with isolate 176 resulted in the highest DSI, higher than treatment with isolate 58, the other isolate tested from AG-5-2. Of the isolates tested belonging to AG-11, the plants treated with isolated 101 had the highest disease severity, albeit closely followed by isolate 16. On average, AG-5-2 had the highest DSI followed by AG-4 HGII and AG-11. However, of most AGs, only one isolate was tested, which means these averages should be interpreted with caution.

Figure 11 shows the means of the weight of the plants in each treatment. Plants treated with isolate 175 were found to weigh significantly less than plants treated with isolates 40, 61 and 176. There were otherwise no significant differences in the weight of the plants founds between treatments. There were also no significant differences observed in the average plant length between treatments, which are given in Figure A 1 in Appendix.

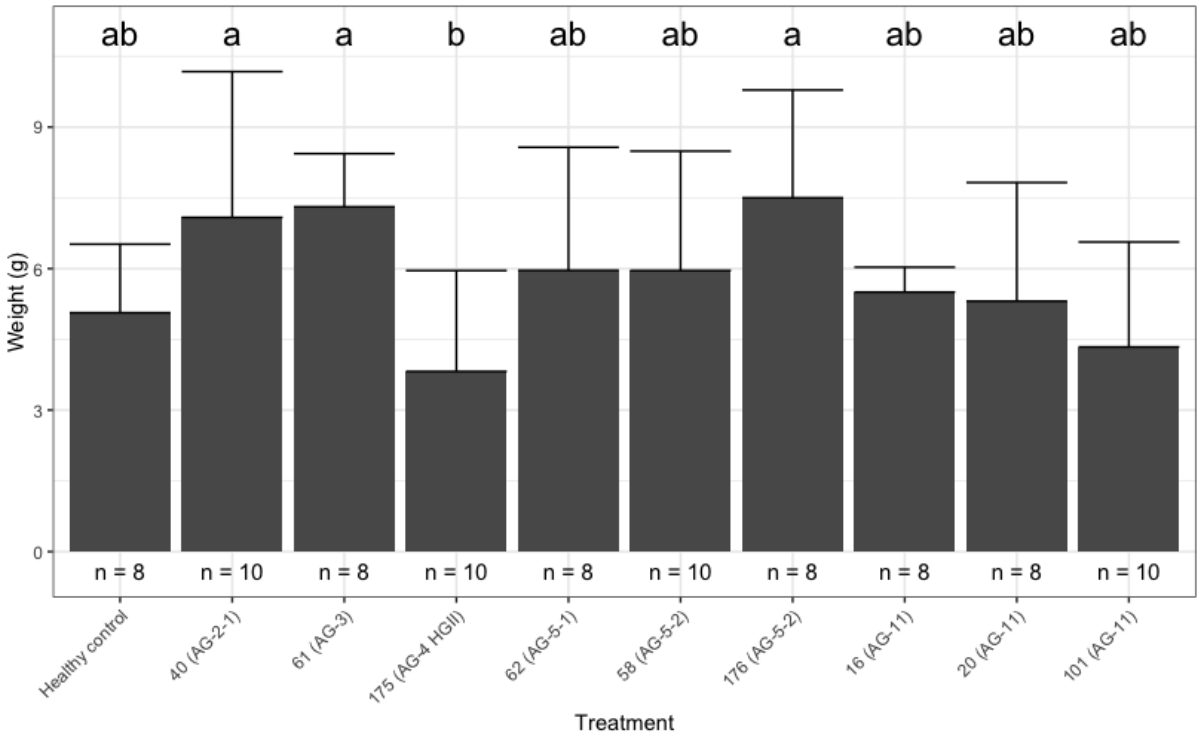


Figure 11. The average weight of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 2. The error bars indicate the standard deviations on the measurements. Differing letters at the top of the graph indicate a statistically significant difference in plant weight between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

Based on the results of plant trial 1 and 2, five isolates belonging to different AGs were chosen for further research during this thesis. Isolates 61 and 175 were chosen since they were the only isolates tested that belonged to AG-3 and AG-4 HGII respectively. From AG-5-1, isolate 139 was chosen since it was found to cause a higher disease severity, although not significantly, and DSI than isolate 62 in plant trial 1. There were also no significant differences in disease severity in both plant trials between the isolates belonging to AG-5-2. Since isolate 58 twice resulted in a relatively high DSI, this isolate

was chosen. Once again, there were no significant differences in disease severity between isolates from AG-11 in both plant trials. Here isolate 16 was chosen following the same reasoning as above, with this isolate twice resulting in a relatively high DSI.

5.4) *In vitro* antagonism of *Pseudomonas* spp. and *Bacillus* spp. against *Rhizoctonia solani* (*in vitro* antagonism test 1)

The goal of this experiment was to evaluate the biocontrol activity of *Bacillus* strain GA1 and *Pseudomonas* strains COR58, RHF3.3-3 and UPB0736 against different isolates and AGs of *R. solani in vitro*. For each isolate separately, this was done by comparing the Percentage Area Inhibition (PAI) the different bacteria caused. This was done four time, namely for each combination of the two different temperatures (20 °C and 28 °C) and two different time points of measurement (three and seven days after incubation). As the analyses of the dual cultures seven days after incubation seemed more relevant, these results will be shown here. The results of the analyses three days after incubation are shown in Figure A 2 and Figure A 3 in Appendix. A selection of pictures from these dual cultures are shown in Table A 1 and Table A 2 in Appendix.

Unfortunately, two contaminations took place, being the dual culture where isolate 139 and strain UPB0736 were incubated at 20 °C and the control plate of isolate 175 that was incubated at 28 °C. These could not be further analyzed and were removed from the experiment, meaning these dual cultures only had three replicates instead of four.

5.4.1) Percentage area inhibition seven days post incubation at 20 °C

The average PAIs of the dual cultures incubated at 20°C, seven days after incubation are shown in Figure 12. Significant differences were found in the growth inhibition of the bacterial strains in each *R. solani* isolate. The growth of isolate 61 (AG-3) was significantly more inhibited by RHF3.3-3 than by COR58 and UPB0736. There were no significant differences in growth inhibition of isolate 61 between bacterial strains RHF3.3-3 and GA1 and between COR58, GA1 and UPB0736. The PAI of isolate 175 (AG-4 HGII) caused by UPB0736 was significantly higher than those caused by COR58 and GA1, although there was no significant difference between UPB0736 and RHF3.3-3. There was also no significant difference in the growth inhibition of isolate 175 as a result of RHF3.3-3 and GA1 coculturing, however both these strains led to a higher growth inhibition than COR58 did. GA1, RHF3.3-3 and UPB0736 all inhibited the growth of isolates 139 (AG-5-1) and 16 (AG-11) significantly more than COR58 did, while not resulting in significantly different PAIs amongst each other. Finally, isolate 58 (AG-5-2) had its growth significantly more inhibited by RHF3.3-3 and UPB0736 than by COR58. For this isolate, there were no significant differences observed between the PAI caused by GA1 and the other bacterial strains.

To further visualize these findings, a selection of pictures from the dual cultures are shown in Table 19. The selected dual cultures were specifically chosen to be representative of all the replicates in a particular setup. Interestingly, quite often the mycelium seems to develop pigmentation, depending on the specific *R. solani* isolate and bacterial strain in the dual culture. In general, a brown color seems to develop in isolates 61 and 175 while a yellow color develops in isolates 139, 58 and 16.

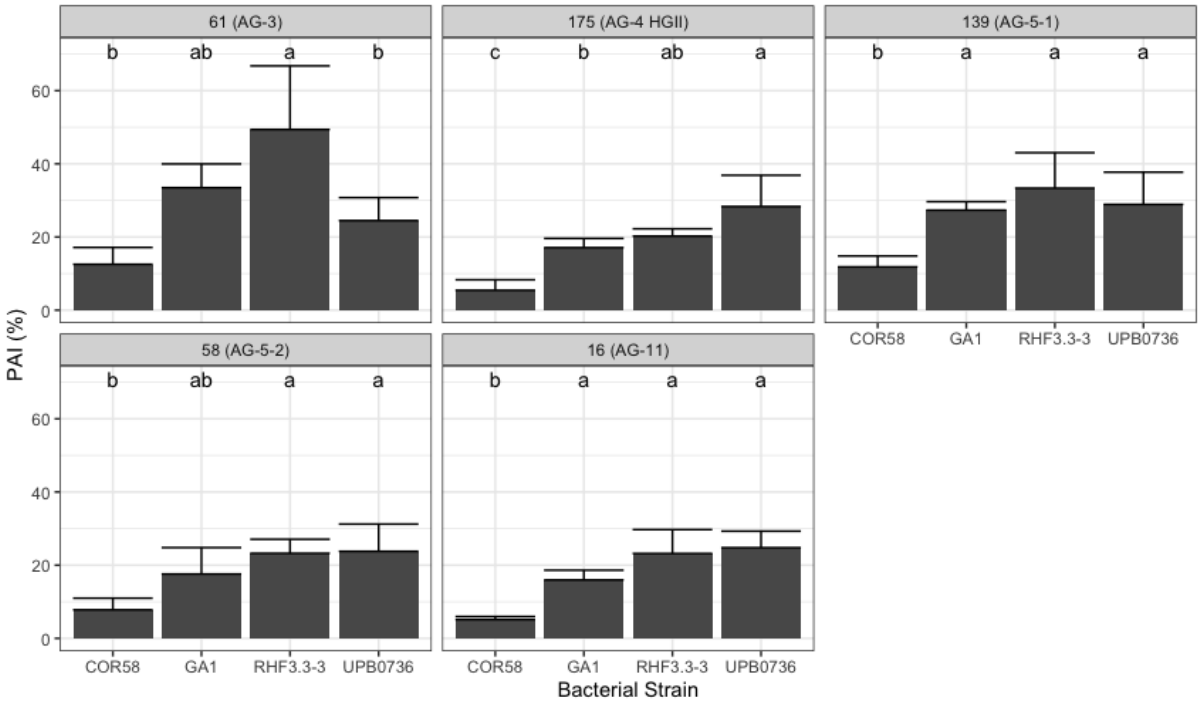
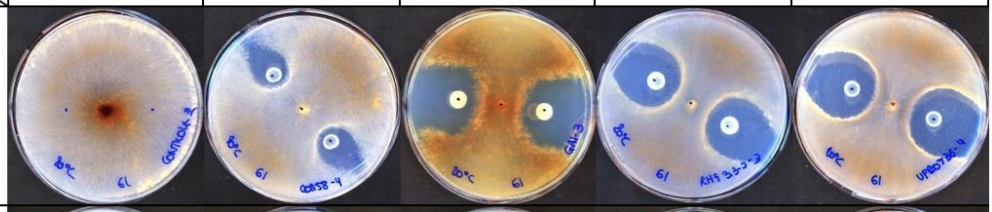


Figure 12. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups seven days after the incubation at 20 °C during *in vitro* antagonism test 1. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. Except for the dual culture of *R. solani* isolate 139 and bacterial strain UPB0736 which had three replicates, all dual cultures and controls had four replicates.

Table 19. A selection of representative pictures taken seven days after incubation at 20 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 1.

Control/ Bacteria / Isolate	Control	COR58	GA1	RHF3.3-3	UPB0736
61 (AG-3)					
175 (AG-4 HGII)					
139 (AG-5-1)					
58 (AG-5-2)					
16 (AG-11)					

5.4.2) Percentage area inhibition seven days post incubation at 28 °C

The average PAIs of the dual cultures at 28 °C, seven days after incubation are shown in Figure 13. Within each isolate except isolate 139 (AG-5-1), there were significant differences in PAI between the different bacterial strains. In the case of isolates 61 (AG-3), 175 (AG-4 HGII) and 58 (AG-5-2), there was always one bacterial strain that resulted in a significantly higher PAI than the others, while the PAI caused by these other bacterial strains did not significantly differ from one another. For isolate 61, the bacterial strain that resulted in the most growth inhibition was GA1. For isolate 175 this was RHF3.3.-3 while for isolate 58 it was found that UPB0736 resulted in the most growth inhibition. Finally, it was found that GA1 and RHF3.3-3 caused a significantly higher growth inhibition of isolate 16 (AG-11) than COR58 and UPB0736 did. There were otherwise no significant differences in PAI of isolate 16.

A selection of representative pictures from the dual cultures are shown in Table 20. These were selected in the same manner as before and the mycelium again seems to develop pigmentation depending on the specific *R. solani* and bacterial strain used.

Because there seems to be notable differences in PAI depending on the specific *R. solani* isolate and temperature, it was difficult to select bacterial strains for further testing *in vivo*. Here GA1 was selected because it was the only *Bacillus* strain tested. *Pseudomonas* strain RHF3.3-3 was selected due to it clearly performing better than COR58 and slightly better overall than UPB0736.

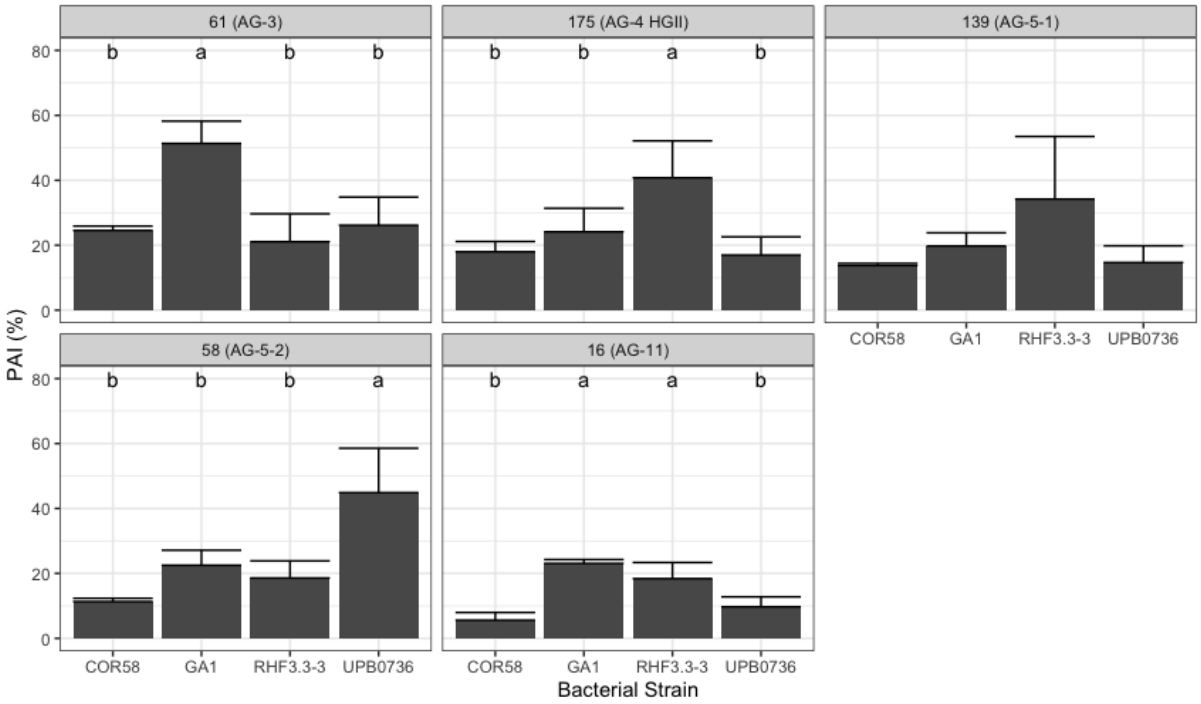
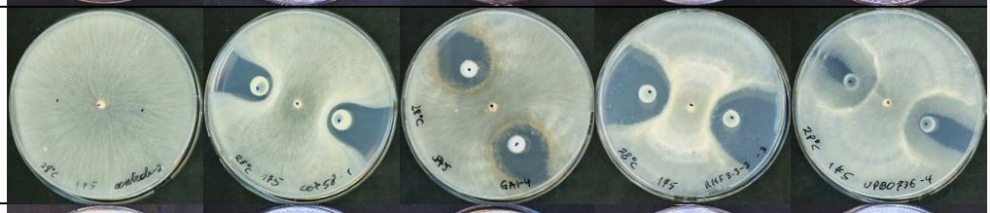


Figure 13. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups seven days after the experimental setup at 28 °C during *in vitro* antagonism test 1. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. Except for the control plate of *R. solani* isolate 175 which had three replicates, all dual cultures and controls had four replicates.

Table 20. A selection of representative pictures taken seven days after incubation at 28 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 1

Control/ Bacteria / Isolate	Control	COR58	GA1	RHF3.3-3	UPB0736
61 (AG-3)					
175 (AG-4 HGII)					
139 (AG-5-1)					
58 (AG-5-2)					
16 (AG-11)					

5.5) Testing of disease control capability of *Bacillus velezensis* strain GA1 and fludioxonil against *Rhizoctonia solani* inoculated *Vicia faba* L. (plant trial 3)

The goal of plant trial 3 was to evaluate the disease control capabilities of *Bacillus subtillis* strain GA1. Disease control by using fludioxonil seed coating was also tested in order to compare the biological control method to a more traditional chemical control. Seeds were sown in three different batches, being a control batch, a batch where the seeds were coated with fludioxonil and a batch where the seeds and substrate were treated with *Bacillus velezensis* strain GA1. The data obtained from plant trial 3 was used to carry out three analyses. Firstly, the data from the control batch was used to again compare the different isolate treatments with one another, as to have a better understanding of the pathogenicity and aggressiveness of the *R. solani* isolates used in this plant trial. Secondly, the number of seeds that germinated in each sowing batch was compared in order to assess if there was any

difference in germination potential between the different batches. This was done twice, once for the number of sprouted plants at the moment of inoculation and once at the moment of evaluation. Finally, the data was used to assess whether GA1 is capable of suppressing the disease incidence of different *R. solani* isolates and AGs in faba bean. This analysis was carried out by, for each isolate, comparing the plants sown in the control batch, the plants sown in the fludioxonil batch and the plants sown in the GA1 batch. The plants were compared using the disease severity, DSI, length and weight of the plants. It should be noted that a couple of diseased plants were observed in the healthy control, specifically one plant sown in the control batch and two plants sown in the fludioxonil batch.

5.5.1) Pathogenicity and aggressiveness

The result of the disease scoring of plants treated with different isolates of *R. solani* and a healthy control in the control batch is given in Figure 14. In this experiment, treatment with isolates 61 (AG-3) and 58 (AG-5-2) did not result in a significantly higher disease severity than the healthy control. Plants treated with isolates 175 (AG-4 HGII), 139 (AG-5-1) and 16 (AG-11) did have a higher disease severity than the healthy control. Treatment with isolates 175 and 16 also resulted in a significantly higher disease severity than treatment with isolates 61, but not treatment with isolate 58 or 139.

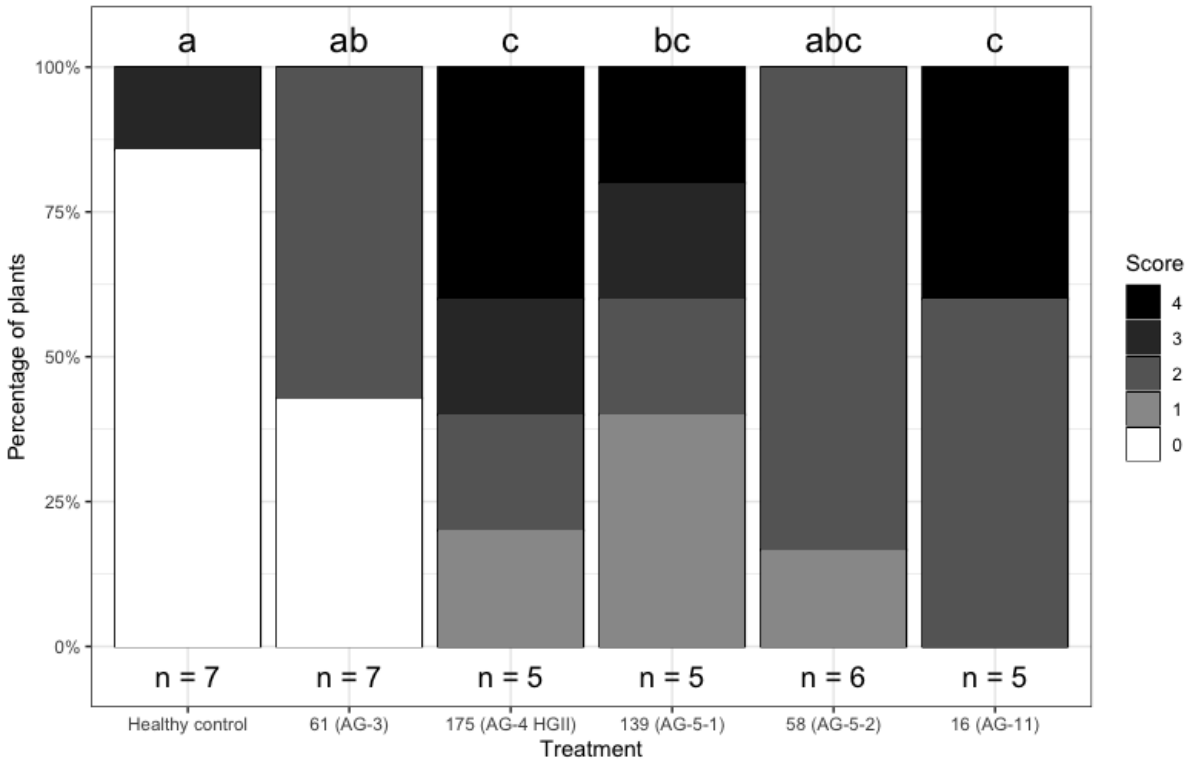


Figure 14. Observed disease severity on faba bean of different *Rhizoctonia solani* isolates and a healthy control in the control batch in plant trial 3, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate a statistically significant difference in disease severity between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

The DSI of each treatment is shown in Table 21. It can again be discerned that from all isolate treatments in the control batch, isolate 61 resulted in the lowest DSI. Plants treated with isolate 58 had a lower DSI than plants treated with isolate 139 but overall, treatments with isolate 175 and 16 resulted in the highest DSIs of all treatments.

Table 21. The disease severity indices on faba bean in the different sowing batches of each *Rhizoctonia solani* isolates and a healthy control in plant trial 3.

Treatment/Isolate	Batch	Disease Severity Index (%)
Healthy control	Control	10.71
	Fludioxonil	29.17
	GA1	0.00
61 (AG-3)	Control	28.57
	Fludioxonil	46.88
	GA1	53.57
175 (AG-4 HGII)	Control	70.00
	Fludioxonil	66.67
	GA1	32.41
139 (AG-5-1)	Control	55.00
	Fludioxonil	32.14
	GA1	21.43
58 (AG-5-2)	Control	45.83
	Fludioxonil	33.33
	GA1	53.57
16 (AG-11)	Control	70.00
	Fludioxonil	46.43
	GA1	68.75

Figure A 4 and Figure A 5 in Appendix show the average plant length and weight respectively, in each treatment of the control batch. No significant differences were found between the treatments.

5.5.2) Germination potential

The average number of germinated plants in each batch at the moment of inoculation and evaluation are given in Figure 15. Significantly less plants had sprouted in the control batch than had in the fludioxonil or GA1 batch at the moment of inoculation. When evaluating the plants, enough plants had sprouted from the control batch as to result in no significant difference between the number of plants in the control and fludioxonil batch. However, there was still a significant difference between the control and GA1 batch.

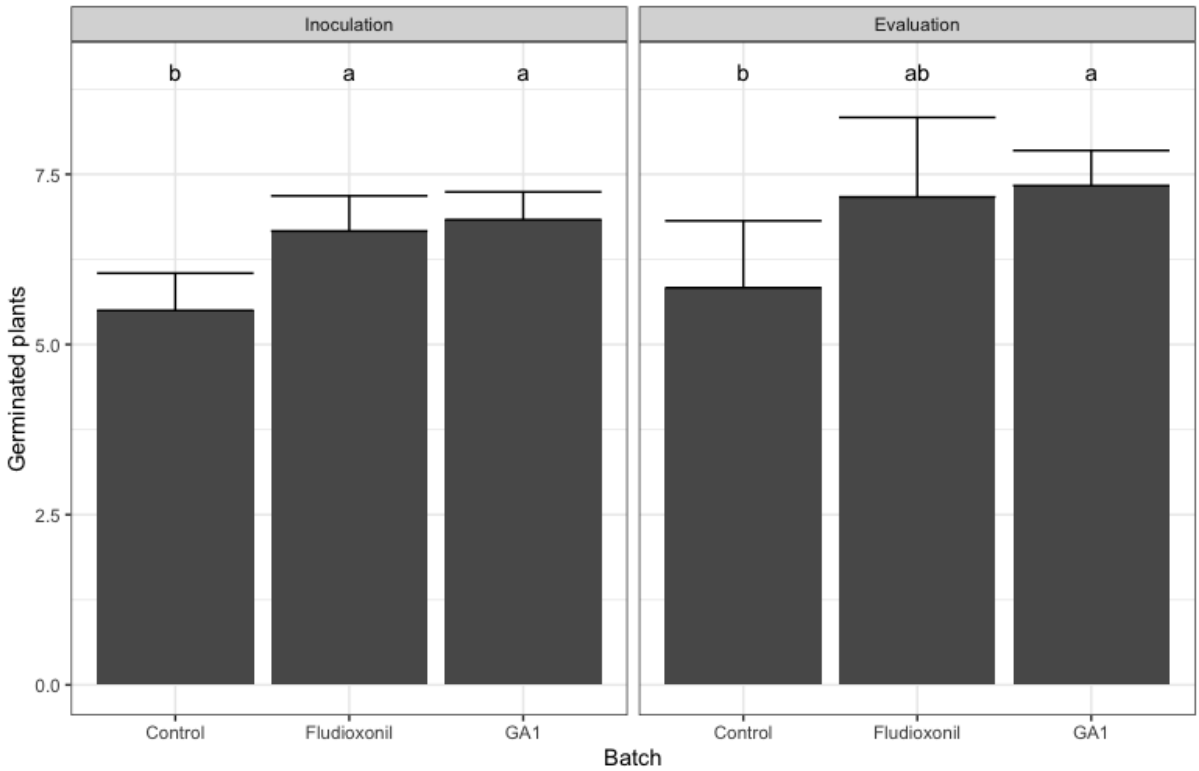


Figure 15. The average number of faba bean plants that sprouted in each batch at the moment of inoculation and evaluation in plant trial 3. The error bars indicate the standard deviations on the data. Differing letters at the top of the graph indicate a statistically significant difference in the number of sprouted plants between batches at each moment.

5.5.3) Disease control of fludioxonil and *Bacillus velezensis* strain GA1

The results of the final analysis are visualized in Figure 16. As can be seen, there were no significant differences in disease severity between the different sowing batches for all isolates except isolate 175. Here, the plants that had been treated with GA1 were significantly less diseased than the plants that had received a seed coating of fludioxonil and the diseased control. Between these last two treatments, no significant difference was found.

When looking at Table 21, it can be noted that both the DSI of the fludioxonil and GA1 batch were higher for isolate 61 than the diseased control. For isolates 175 and 139, the GA1 treatment resulted in a lower DSI of the plants than the fludioxonil treatments, which was still lower than the DSI of the diseased controls. For the isolates 58 and 16 this was the other way around, with fludioxonil treated plants having a lower DSI than the GA1 treated plants and diseased control. In the case of plants treated with isolates 58, the DSI of the GA1 batch even was higher than the DSI of the diseased control.

Figure A 6 and Figure A 7 in Appendix show the means in each treatment of the length and weight of the plants respectively. However, there were no significant differences found within each isolate treatment between the different sowing batches in either the length or weight of the plants. The weight measurements of the healthy control were found to be heteroscedastic, hence prompting the use of the Brown-Forsythe test.

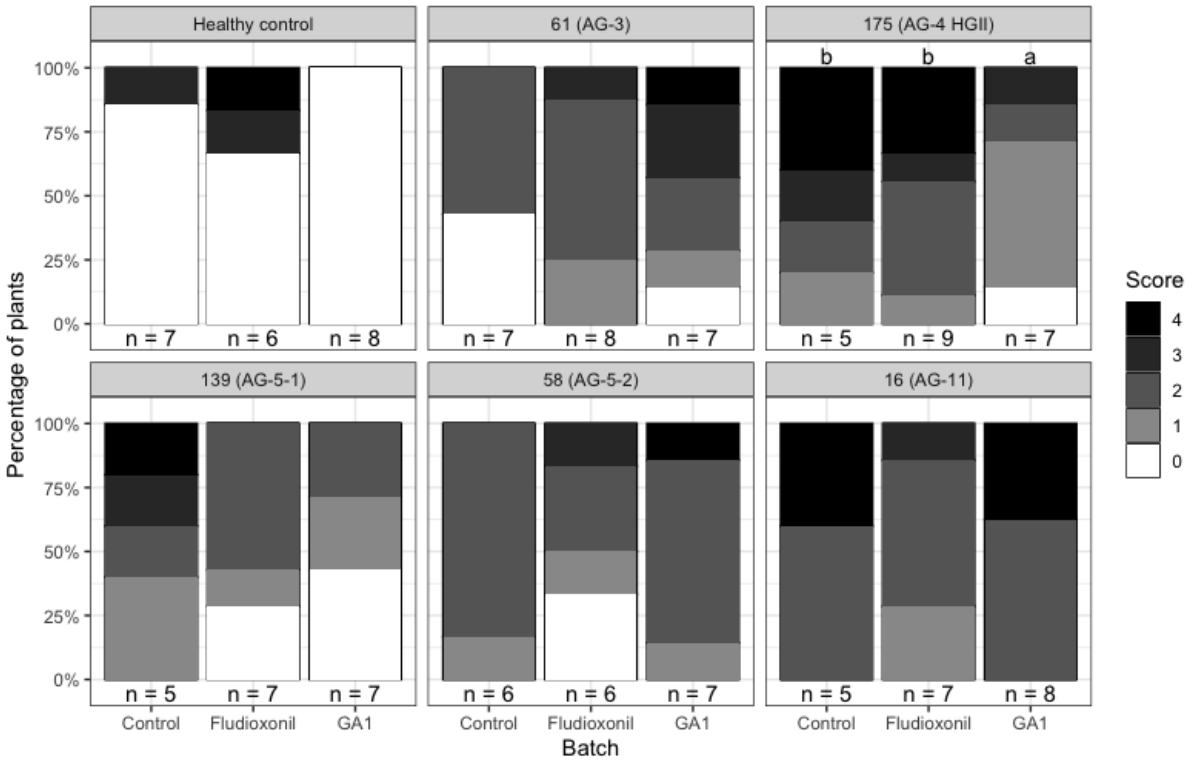


Figure 16. Observed disease severity on faba bean in the different sowing batches for each *Rhizoctonia solani* isolate and a healthy control in plant trial 3, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate that within an isolate treatment, there were statistically significant differences in disease severity between the different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

5.6) Testing of disease control capability of *Pseudomonas asplenii* G1 strain RHF3.3-3 and fludioxonil against *Rhizoctonia solani* inoculated *Vicia faba* L. (plant trial 4)

The previous experiment was repeated in plant trial 4, now testing the biocontrol activity of *Pseudomonas asplenii* G1 strain RHF3.3-3 against *R. solani* instead of *Bacillus velezensis* strain GA1. Again, the pathogenicity and aggressiveness of the isolate treatments in the control batch were assessed first after which the germination potential of the different sowing batches was evaluated. Finally, the disease control capabilities of RHF3.3-3 was compared with a chemical control using fludioxonil seed coatings and a diseased control. Again, it must be noted that some diseased plants were observed in the healthy control, specifically one in the control batch and one in the RHF3.3-3 batch.

5.6.1) Pathogenicity and aggressiveness

The results of the disease scoring in the control batch of plants treated with different isolates of *R. solani* and a healthy control are given in Figure 17. This time, only the plants that were treated with isolate 175 (AG-4 HGII) had a significantly higher disease severity than the healthy control. These plants were also significantly more diseased than plants treated with isolates 61 (AG-3) and 139 (AG-5-1), but not the plants treated with isolate 58 (AG-5-2).

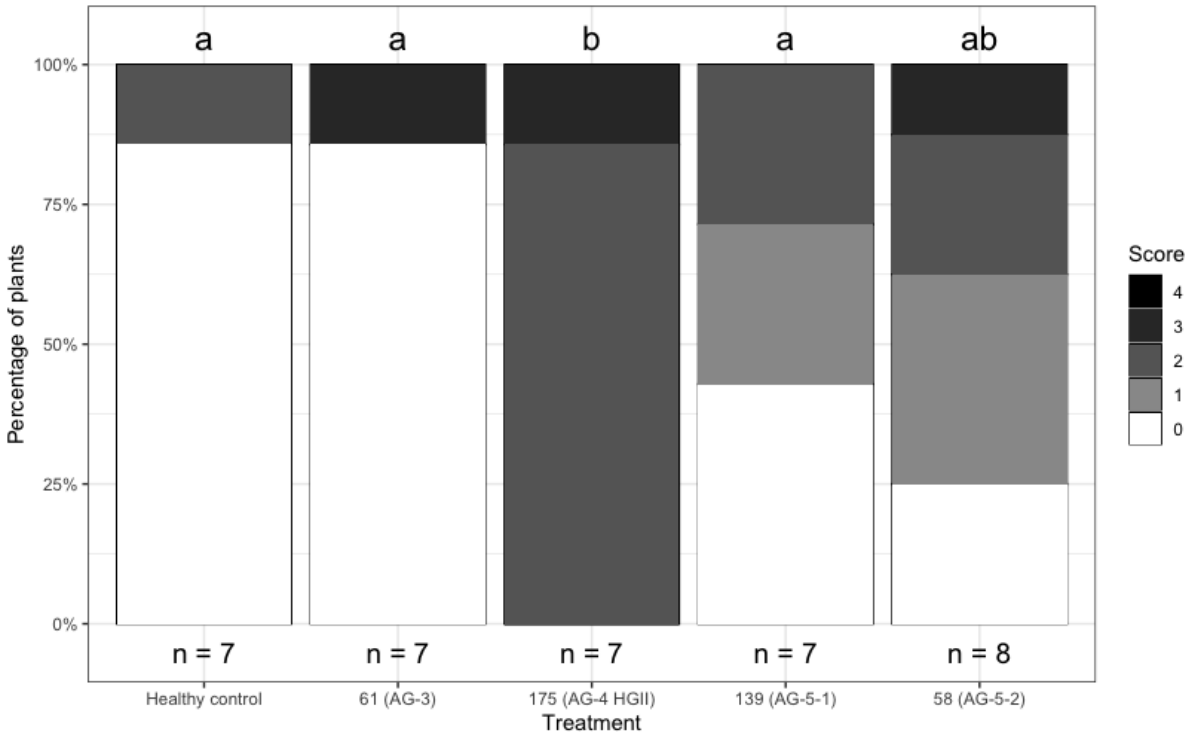


Figure 17. Observed disease severity on faba bean of different *Rhizoctonia solani* isolates and a healthy control in the control batch in plant trial 4, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate a statistically significant difference in disease severity between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

The same as stated above can be discerned from Table 22, which shows the DSI of each treatment. In the control batch, treatment with isolate 175 results in the highest DSI. Although this time the DSI of plants treated with isolate 58 is higher than that of plants treated with isolate 139, they are both relatively low. Treatment with isolates 61 again resulted in the lowest DSI out of all the isolate treatments in the control batch and is barely higher than the DSI of the healthy control.

The means of the plant length and weight in each treatment in the control batch are shown in Figure A 8 and Figure A 9 in Appendix. These were relatively consistent between treatments, resulting in no significant differences.

Table 22. The disease severity indices on faba bean in the different sowing batches of each *Rhizoctonia solani* isolates and a healthy control in plant trial 4.

Treatment/Isolate	Batch	Disease Severity Index (%)
Healthy control	Control	7.14
	Fludioxonil	0.00
	RHF3.3-3	10.00
61 (AG-3)	Control	10.71
	Fludioxonil	22.22
	RHF3.3-3	25.00
175 (AG-4 HGII)	Control	53.57
	Fludioxonil	37.50
	RHF3.3-3	60.00
139 (AG-5-1)	Control	21.43
	Fludioxonil	13.89
	RHF3.3-3	31.25
58 (AG-5-2)	Control	31.25
	Fludioxonil	18.75
	RHF3.3-3	31.25

5.6.2) Germination potential

Figure 18 shows the average of the number of plants that had sprouted in plant trial 4 at the moment of inoculation and evaluation. There were significant differences between the three batches at both timepoints. The fludioxonil batch twice had a significantly higher number of sprouted plants than the control batch, which in turn twice had a significantly higher number of sprouted plants than the RHF3.3-3 batch.

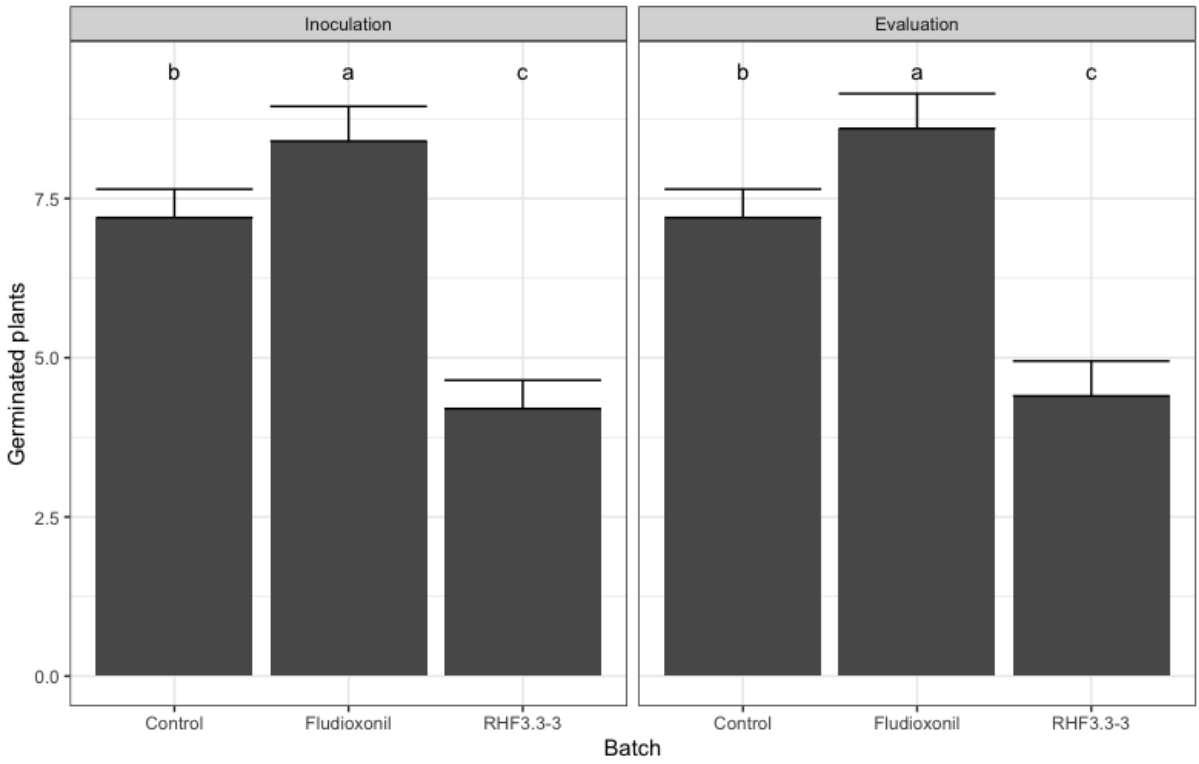


Figure 18. The average number of faba bean plants that sprouted in each batch at the moment of inoculation and evaluation in plant trial 4. The error bars indicate the standard deviations on the data. Differing letters at the top of the graph indicate a statistically significant difference in the number of sprouted plants between batches at each moment.

5.6.3) Disease control of fludioxonil and *Pseudomonas asplenii* G1 strain RHF3.3-3

The evaluation of the disease control capabilities of fludioxonil and RHF3.3-3 are shown in Figure 19. As in the previous experiment, there were no significant differences in disease severity between the different sowing batches for all isolates except isolate 175. This time, the plants that had been treated with RHF3.3-3 were significantly more diseased than those that had received a seed coating of fludioxonil, but this difference was not significant compared to the diseased control. The fludioxonil treatment also did not result in significantly less disease severity compared to the diseased control.

When comparing the different DSIs listed in Table 22, it can again be observed that the DSIs of the fludioxonil and RHF3.3-3 batches were higher than the control batch when plants were treated with isolate 61. When treating the plants with isolates 175 and 139, the DSI was lower when the seeds were coated with fludioxonil compared to the diseased control but also higher when the seeds were treated with RHF3.3-3. Similarly, plants inoculated with isolate 58 had a lower DSI in the fludioxonil batch but a DSI equal to the diseased control in the RHF3.3-3 batch.

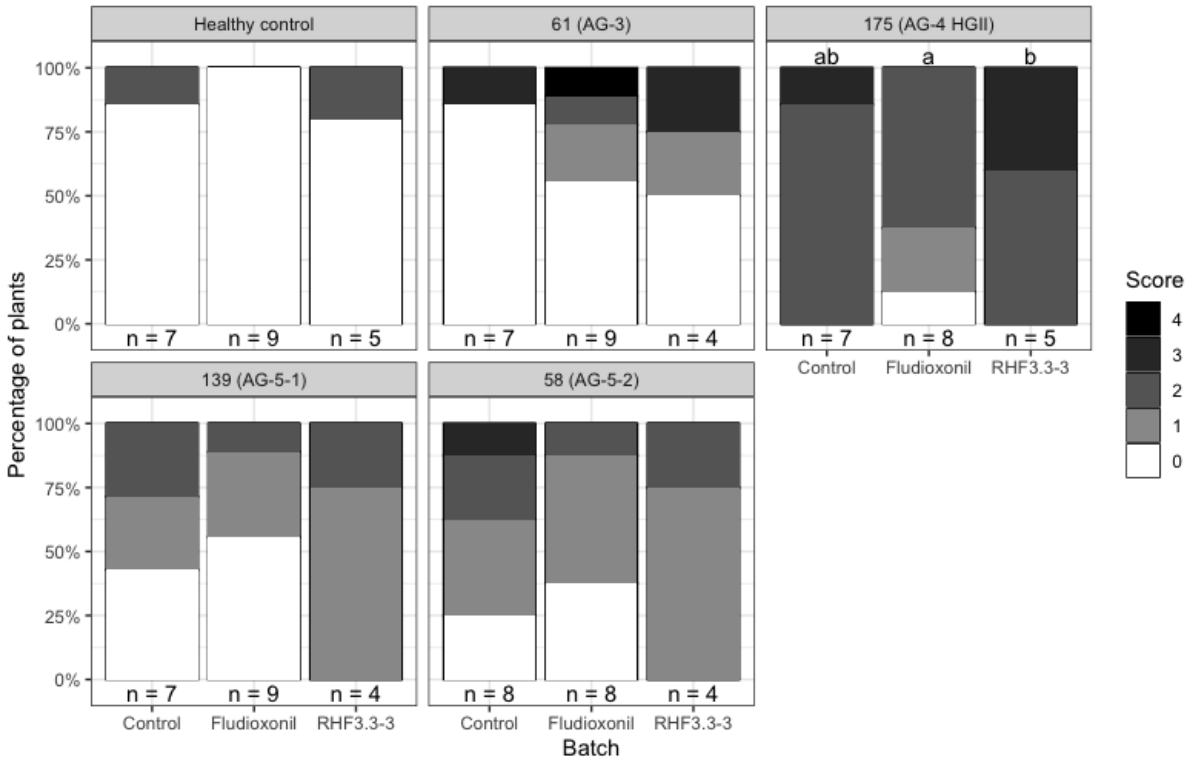


Figure 19. Observed disease severity on faba bean in the different sowing batches for each *Rhizoctonia solani* isolate and a healthy control in plant trial 4, visualized by the percentage of plants that received a disease score of 0 to 4 in each treatment. Differing letters at the top of the graph indicate that within an isolate treatment, there were statistically significant differences in disease severity between the different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

Figure 20 and Figure 21 show the means in each treatment of the length and weight of the plants respectively. As was similarly the case for the disease severity, there are only significant differences for the plants treated with isolate 175. These plants were significantly shorter and weighed less when treated with RHF3.3-3 compared to those plants treated with fludioxonil. They were also significantly lighter when treated with RHF3.3-3 than the plants sown in the control batch but not significantly shorter than these plants.

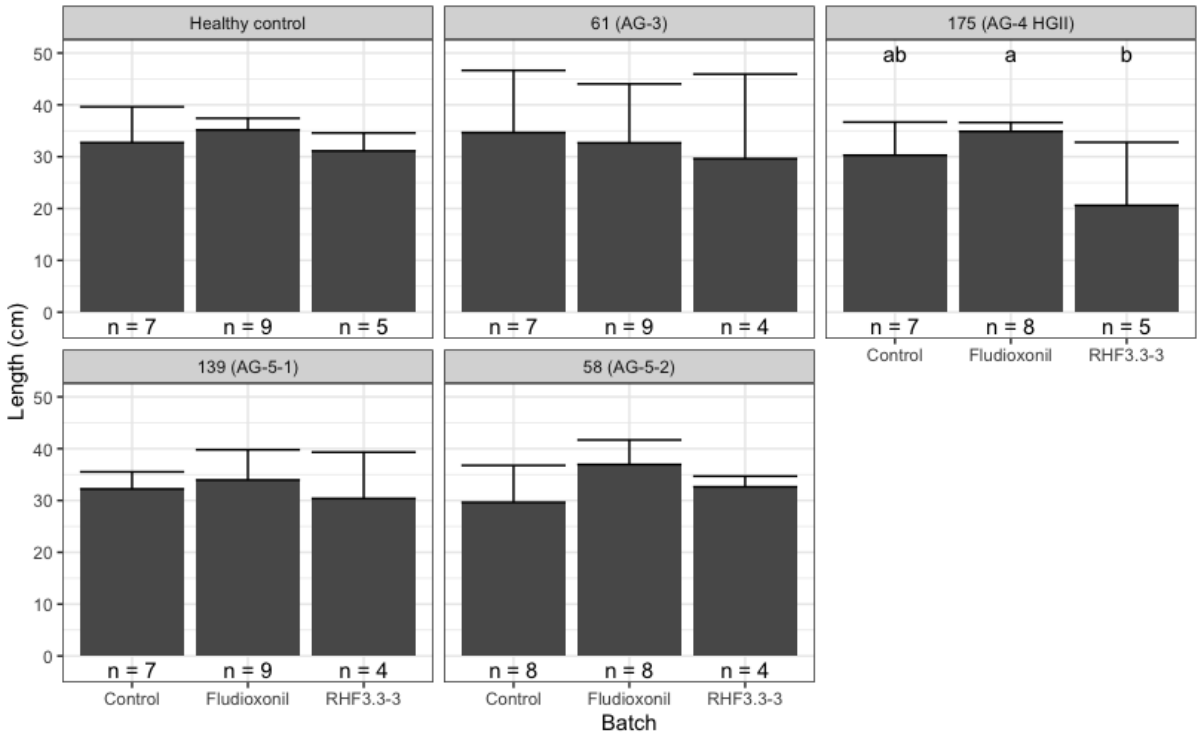


Figure 20. The average length of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 4. The error bars indicate the standard deviations on the measurements. Differing letters at the top of the graph indicate that within an isolate treatment, there were statistically significant differences in plant length between the different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

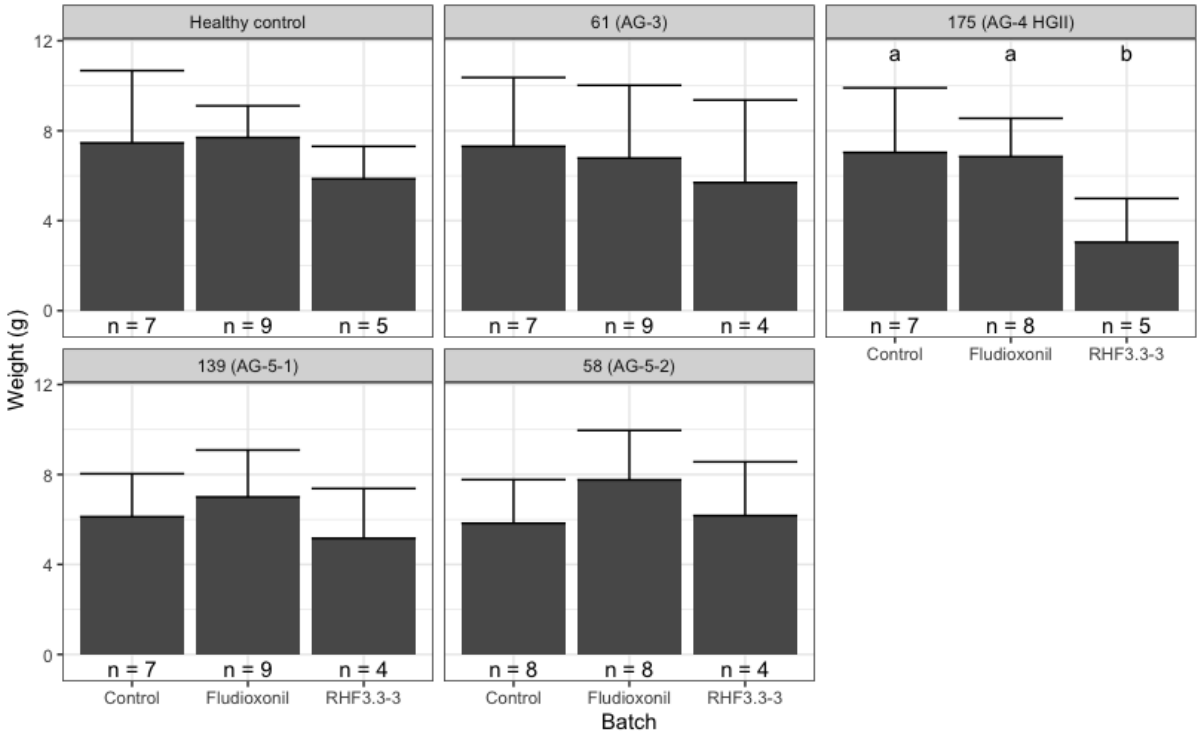


Figure 21. The average weight of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 4. The error bars indicate the standard deviations on the measurements. Differing letters at the top of the graph indicate that within an isolate treatment, there were statistically significant differences in plant weight between the different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

5.7) *In vitro* effectiveness of fludioxonil against *Rhizoctonia solani* (*in vitro* fludioxonil test)

Due to fludioxonil surprisingly not being very effective against *R. solani* in the last two plant trials, an *in vitro* experiment testing the effectiveness of fludioxonil against different *R. solani* isolates was conducted. This was done by examining the growth inhibition (GI) of different concentrations of fludioxonil on these isolates. Using this data, EC_{50} values of fludioxonil of each *R. solani* isolate were estimated. These values were then compared between isolates to assess which isolates were more susceptible to fludioxonil, lower EC_{50} values indicating a higher susceptibility. The average EC_{50} values of fludioxonil are given in Figure 22.

The isolate that was most susceptible to growth inhibition due to fludioxonil was isolate 61 (AG-3), having a significantly lower EC_{50} than all other isolates. Isolates 62 (AG-5-1) and 175 (AG-4 HGII) have the second and third lowest EC_{50} value of all isolates tested. These are also significantly lower than all other isolates except for isolate 61. The isolate most tolerant to fludioxonil was isolate 176 (AG-5-2), having the highest EC_{50} value that is also significantly higher than all other estimated EC_{50} values. The other isolates all had similar EC_{50} values around 0.10 mg/L and no other statistical differences were observed.

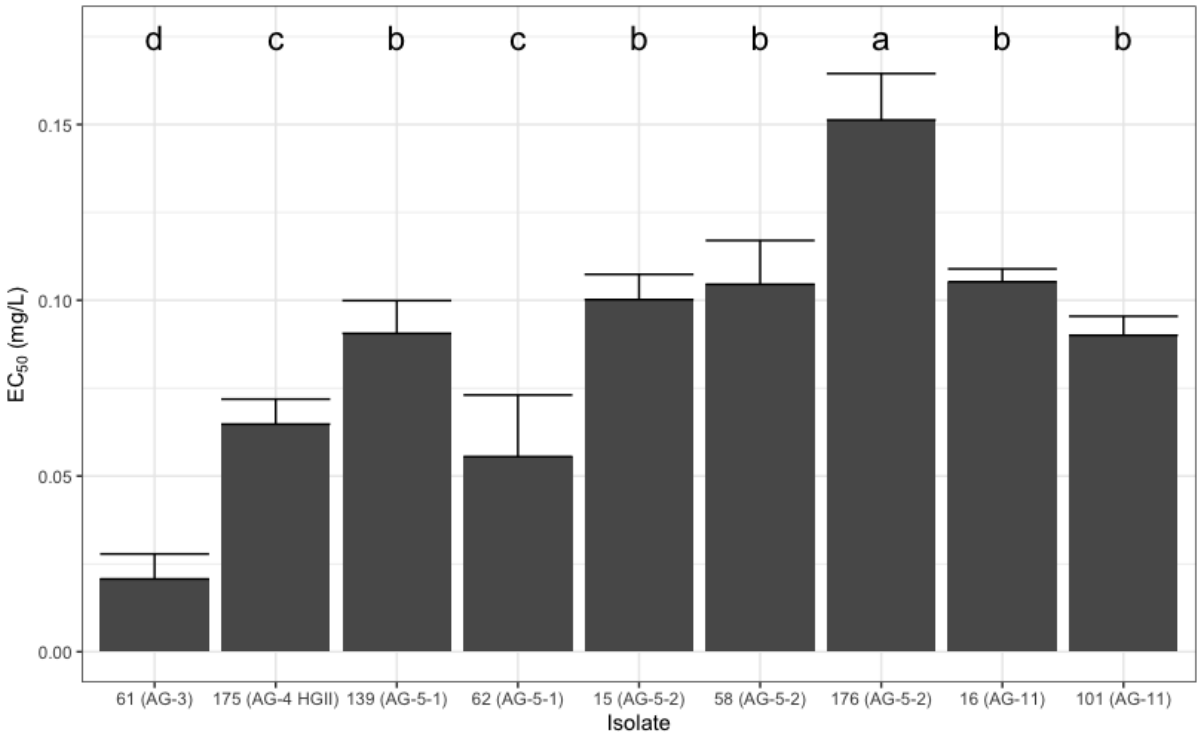


Figure 22. Average half maximal effective concentration (EC_{50}) of fludioxonil against *Rhizoctonia solani* isolates belonging to different anastomosis groups, estimated on the basis of the growth inhibition measurements of the *in vitro* fludioxonil test. The error bars indicate the standard deviation on the estimations. Differing letters at the top of the graph indicate a statistically significant difference in EC_{50} values between isolates. Four estimations were made for each isolate based on the seven combinations of *R. solani* isolate and fludioxonil concentrations.

5.8) *In vitro* antagonism of *Bacillus velezensis* strain GA1 mutants against *Rhizoctonia solani* (*in vitro* antagonism test 2)

Since GA1 seemed to work best as a biocontrol agent against *R. solani* on faba bean, the *in vitro* antagonism test was repeated, but with mutants of GA1. These mutants are only capable of producing one type of CLiP (either iturin, surfactin or fengycin). The goal was to examine which CLiP(s) produced by GA1 is/are the most important for biocontrol activity of the bacteria against *R. solani*. The types of CLiPs produced by the different bacterial strains is listed in Table 4 (section 4.1.1). Again, four analyses were carried out, one for each combination of the examined temperatures (20 °C and 28°C) and time points of measurement (three and seven days after incubation). The analyses are also the same as before, looking at each *R. solani* isolate separately and comparing the PAI of the different bacteria used. Again, only the analyses of the dual cultures seven days after incubation will be shown here. The results of the analyses three days after incubation are shown in Figure A 10 and Figure A 11 in Appendix. A selection of pictures from these dual cultures are shown in Table A 3 and Table A 4 in Appendix.

Unfortunately, two contaminations of the isolate 58 and GA1ΔfenA-ituA dual culture incubated at 28 °C and one contamination of the isolate 58 and GA1ΔsrfaA-ituA dual culture took place incubated at 20 °C, meaning they were left out of the analyses. These dual cultures had only two and three replicates respectively.

5.8.1) Percentage area inhibition seven days post incubation at 20 °C

Figure 23 shows the average PAI of the dual cultures incubated at 20°C, seven days after incubation. Wild type GA1 and GA1ΔsrfaA-ituA resulted in a significantly higher growth inhibition of isolate 61 (AG-3) than GA1ΔfenA-ituA did. The PAI of the dual cultures of GA1ΔsrfaA-fenA and isolate 61 did not have a significantly different PAI than the other dual cultures of isolate 61. Dual cultures of isolates 175 (AG-4 HGII), 139 (AG-5-1), 58 (AG-5-2) and 16 (AG-11) all behaved in the same manner, with wild type GA1 and the iturin producing GA1ΔsrfaA-fenA always having a significantly higher growth inhibition of *R. solani* than GA1ΔsrfaA-ituA and GA1ΔfenA-ituA. There were no other significant differences in PAI in these dual cultures.

These findings are visualized using a selection of representative pictures from the dual cultures, shown in Table 23. Again pigmentation occurs, depending on the specific *R. solani* isolate and bacterial strain in the dual culture.

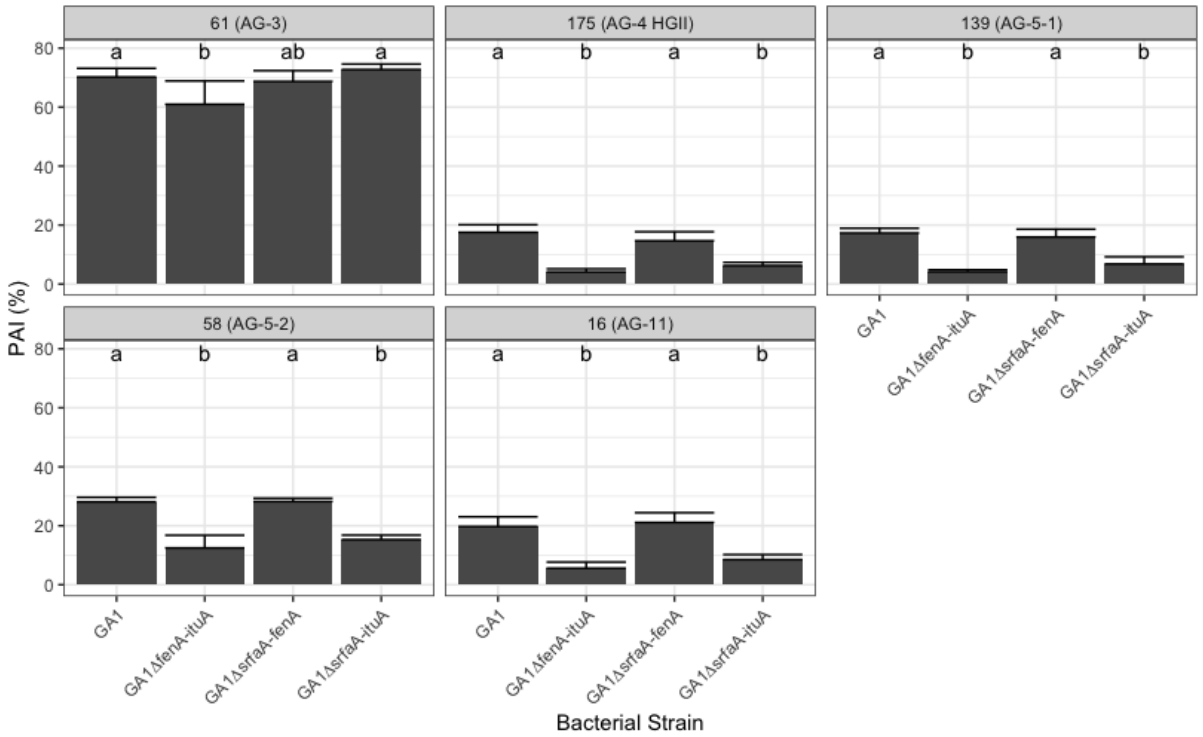


Figure 23. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups seven days after the experimental setup at 20 °C during *in vitro* antagonism test 2. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. Except for the dual culture of *R. solani* isolate 58 and bacterial strain GA1ΔsrfaA-ituA which had three replicates, all dual cultures and controls had four replicates.

Table 23. A selection of representative pictures taken seven days after incubation at 20 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 2.

Control/ Bacteria / Isolate	Control	GA1	GA1ΔfenA-ituA	GA1ΔsrfaA-fenA	GA1ΔsrfaA-ituA
61					
175					
139					
58					
16					

5.8.2) Percentage area inhibition seven days post incubation at 28 °C

The average PAI of all dual cultures at 28 °C, seven days after incubation is shown in Figure 24. There were no significant PAI differences found in the dual cultures of isolates 61 and 175. The same significant difference in PAI as were found seven days post experimental setup at 20°C, were found here in the dual cultures of isolates 139 and 16, where wild type GA1 and GA1ΔsrfaA-fenA resulted in a significantly higher PAI than the non-iturin producing bacterial strains did. When isolate 58 was cocultured with bacterial strains GA1 and GA1ΔsrfaA-fenA, it also resulted in a significantly higher growth inhibition then when the isolate was cocultured with GA1ΔsrfaA-ituA. There was however no significant difference when GA1ΔfenA-ituA was cocultured with isolate 58.

A visualization of the dual cultures is given in Table 24. As previously observed, the mycelium shows pigmentation, depending on the *R. solani* isolate and bacterial strain in the dual culture.

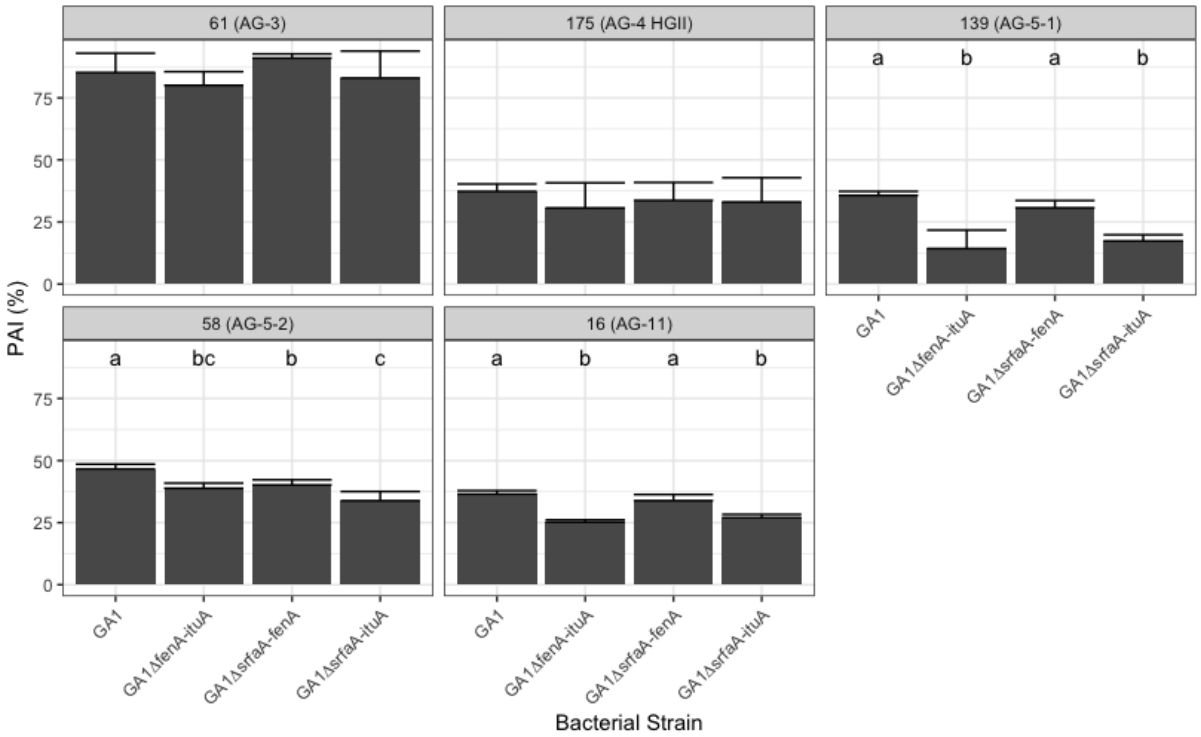
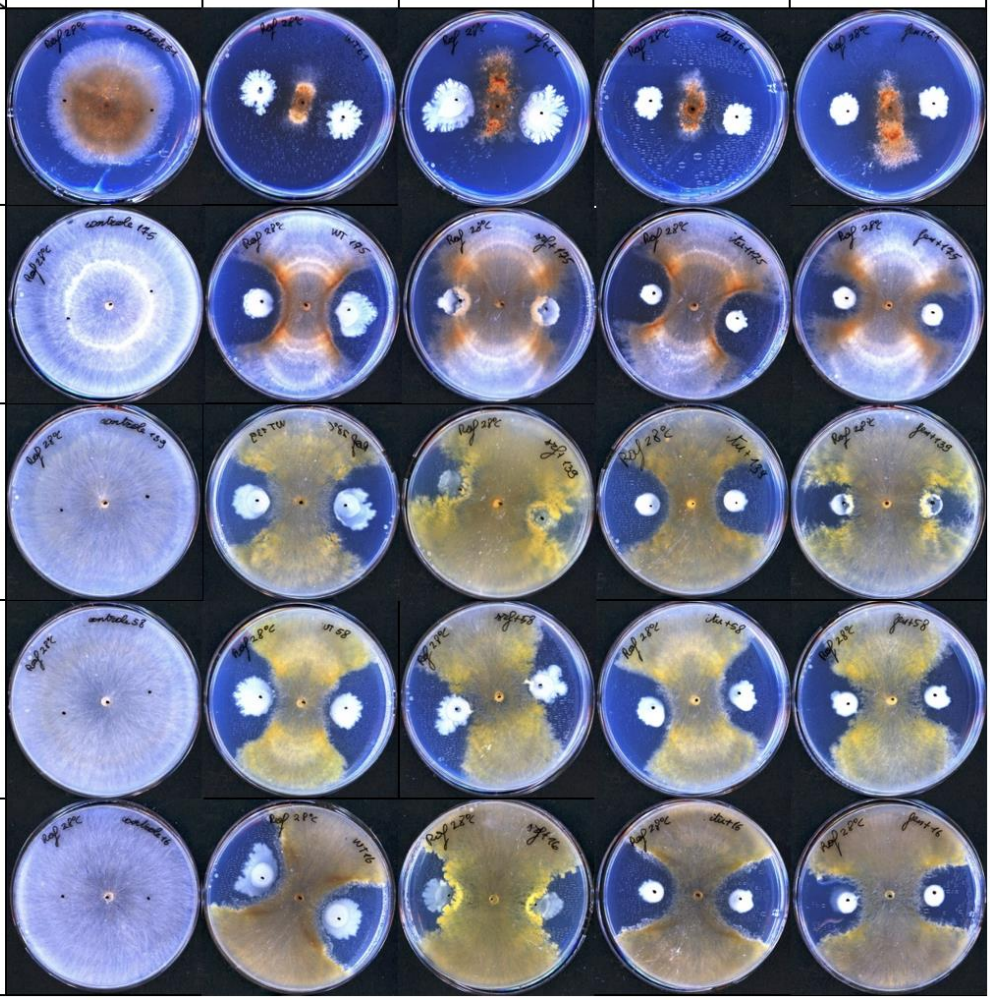


Figure 24. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups seven days after the experimental setup at 28 °C during *in vitro* antagonism test 2. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant statistical differences in PAI between bacterial strains. Except for the dual culture of *R. solani* isolate 58 and bacterial strain GA1ΔfenA-ituA which had two replicates, all dual cultures and controls had four replicates.

Table 24. A selection of representative pictures taken seven days after incubation at 28 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 2.

Control/ Bacteria / Isolate	Control	GA1	GA1ΔfenA-ituA	GA1ΔsrfaA-fenA	GA1ΔsrfaA-ituA
61					
175					
139					
58					
16					

6) Discussion

In the following, the results obtained during this thesis are discussed. Firstly, the observations made during the anastomosis microscopy test are interpreted and compared to previous DNA analysis. Secondly, the pathogenicity and aggressiveness of the different *R. solani* isolates and AGs is assessed. Finally, the effectiveness of the different bacterial strains and fludioxonil in controlling *R. solani* is evaluated. This is done by first evaluating the *in vitro* growth inhibition of *R. solani* caused by different *Bacillus* and *Pseudomonas* strains, after which the disease control potential of these bacteria and fludioxonil on faba bean is assessed. The last two parts consist of evaluating the *in vitro* effectiveness of fludioxonil and determining which CLiP(s) produced by *Bacillus* strain GA1 is/are the most important for its antifungal activity.

6.1) Relatedness of *Rhizoctonia solani* isolates

This section will discuss the results of the anastomosis microscopy test, where the pairings of isolates belonging to different AGs were evaluated to determine possible anastomosis. Based on the classification system of Carling et al. (1988) (Table 1, section 2.2.2), three observations can be made in this experiment, being that I) isolate 175 from AG-4 HGII seemingly is not related to isolates belonging to AG-5 and AG-11, II) AG-5 and AG-11 seem to be distantly related and III) isolates from AG-5-1 and AG-5-2 appear to belong to the same AG but different VCPs.

A phylogenetic study conducted by Abbas et al. (2022) seems to support the first two findings. In this study, isolates belonging to seven AGs were classified as belonging to specific clades and subclades based on genes from the rDNA ITS1-5.8S-ITS2 region. AG-5 and AG-11 were classified as belonging to the same subclade while AG-4 belonged to a different subclade, although these three AGs still belonged to the same clade. Marcou et al. (2021) also conducted a study on these same genes which again concluded that AG-5 and AG-11 are more closely related to each other than they are to AG-4. This was again concluded in unpublished work conducted at the lab of phytopathology at Ghent University, which used many of the same isolates as were used in this experiment and also conducted a sequences analysis on the same genes (Marcou, unpublished).

In this last study, it was also found that AG-5 could be divided into two subgroups, being AG-5-1 and AG-5-2 (Marcou, unpublished). This experiment seems to further support these findings, being that C2 reactions occurred between isolates of the different subgroups and C3 reactions occurred between isolates of the same subgroup. As previously mentioned, Carling et al. (1988) suggest that isolates exhibiting C2 reactions indicate a more distant relatedness than isolates exhibiting C3 reactions, while still belonging to the same AG. Similar anastomosis reactions occur in AG-3, where isolates belonging to the same subgroup display C3 reactions while isolates belonging to different subgroups of AG-3 exhibit C2 reactions (Kuninaga et al., 2000).

6.2) Pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates and anastomosis groups on *Vicia faba* L.

This section discusses the pathogenicity and aggressiveness of the different *R. solani* isolates and AGs based on the disease severity scoring system (Table 9 and Figure 7, section 4.3.4). This was studied during the four plant trials. An overview of the statistical analyses is shown in Table 25. Firstly, a couple of general remarks should be made about the plant trials. Except for plant trial 2, diseased plants were always observed in the healthy control. This is likely due to the presence of another pathogen. If the pathogen originated from the seeds, this must have been from inside the seeds since the outside was sterilized in each plant trial. Since this seems rather unlikely, the pathogen probably originated from the substrate that was used, which was not sterilized beforehand. No analysis was conducted to determine which pathogen was present, but it seems likely that this influenced the results of these experiments, possibly even interacting with *R.solani*. That is why the results of the plant trials should be interpreted with caution, especially plant trial 1 where a substantial number of diseased plants were observed in the healthy control. Secondly, the experiments were set up in such a way that the plants had already germinated when the substrate was inoculated with *R. solani*. This way roughly the same number of plants could be used in each treatment, since plant pots where the seeds did not germinate could be accounted for. This means that symptoms of *R. solani* infection such as seed rot and pre-emergence damping-off could not be accounted for. This is relevant because these have been reported symptoms of *R. solani* on faba bean, specifically of AG-2, AG-4 and AG-5 which were all used in this thesis (Rashid & Bernier, 1993). Finally, it should be noted that the inoculum of plant trial 4 did not seem to grow as well as in the other plant trials, with the seeds being noticeably less covered with mycelium than in the other plant trials. This is a potential reason why the pathogenicity and overall disease severity in plant trial 4 was lower than in the other plant trials.

Table 25. Summary of the statistical data analysis performed on the disease severity scoring to determine the pathogenicity and aggressiveness of different *Rhizoctonia solani* isolates. The disease severity indices (DSIs) of each plant trial are given and rounded to the nearest whole number. Differing letters indicate a statistically significant difference in disease severity between treatments in each plant trial. Colors were assigned using conditional formatting in Excel (Version 16.73 (23051401)), red indicating high disease severity and green indicating low disease severity.

Anastomosis group (AG)	Isolate/ treatment	Plant trial 1	Plant trial 2	Plant trial 3	Plant trial 4
/	Healthy control	23 a	0 a	11 a	7 a
AG-2-1	40		38 bc		
AG-3	61		22 ab	29 ab	11 a
AG-4 HGII	175	29 ab	55 cd	70 c	54 b
AG-5-1	62	42 bc	44 bcd		
	139	48 bc		55 bc	21a
AG-5-2	15	50 c			
	58	50 c	55 cd	46 abc	31 ab
	176	41 bc	59 d		
AG-11	16	56 c	56 cd	70 c	
	20	40 bc	44 bcd		
	101	38 bc	38 d		

When defining pathogenicity as having a significantly higher disease severity than a healthy control, only one isolates was never observed to be pathogenic towards faba bean, being isolate 61 belonging

to AG-3. Isolate 61 was included in three plant trials where treatment of the plants with isolate 61 always resulted in the lowest DSI of all isolates and a disease severity that was significantly lower than isolates belonging to AG-4 HGII, AG-5-2 and AG-11. Isolate 40 of AG-2-1, while being found to be pathogenic, was also not very aggressive. The isolate was only tested once in plant trial 2, where it resulted in the second lowest DSI of all isolates and a significantly lower disease severity than isolates 176 of AG-5-2 and 101 of AG-11. Plants infected with the isolates 40 and 61 even had a higher plant weight than the healthy control and significantly higher than the plants infected with isolate 175 of AG-4 HGII did in plant trial 2. In contrast with these findings, scientific literature does suggest that *R. solani* isolates belonging to AG-2-1 and AG-3 are capable of infecting faba bean (Djébali et al., 2014; Mwiindilila, 1984; H. Yu et al., 2022). It should be noted that only one of each AG was tested here,

Isolate 175 of AG-4 HGII was found to be pathogenic in all but one plant trial, being plant trial 1. As mentioned previously, this can be due to possible interactions with another pathogen present in the substrate. When looking at the other plant trials, isolate 175 was found to be quite aggressive on faba bean, twice resulting in the highest DSI of all isolates. In these plant trials, isolate 175 always resulted in a significantly higher disease severity than isolate 61 of AG-3 and in plant trial 4, it was also higher than disease severity as a result of isolate 139 of AG-5-1. Plants treated with isolate 175 were also twice found to be the lightest plants of a plant trial, namely in plant trial 2 and 3. There even was a significant difference observed between the weight of plants treated with isolate 175 and three other isolates in plant trial 2. This is possibly due to symptoms of *R. solani* like reduced growth and stunting (Djébali et al., 2014; Mahmoud et al., 2007). Even though the number of studies of *R. solani* infections on faba bean is limited, isolates belonging to AG-4 have multiple times been found to be pathogenic towards faba bean (Akladious et al., 2019; Assunção et al., 2011; Azimi et al., 2005; Mohamed et al., 2014a, 2014b, 2015; Mwiindilila, 1984; Rashid & Bernier, 1993; H. Yu et al., 2022).

Two isolates belonging to AG-5-1 were tested, being isolates 62 and 139. Except for isolate 139 in plant trial 4, these were always found to be pathogenic towards faba bean. As explained above, the inoculum seeds in plant trial 4 were noticeably less covered with mycelium, which is a possible explanation for the disease severity of plants treated with isolate 139 not being significantly higher than the healthy control in plant trial 4. Even though these isolates were consistently pathogenic, they were not more aggressive than other isolates, never resulting in a higher disease severity than any other isolate and having an average DSI of roughly 40%.

Three isolates of AG-5-2 were tested, being isolates 15, 58 and 176. When these isolates were tested in the first two plant trial, they were always found to be pathogenic towards faba bean. The level of aggressiveness does seem to vary between plant trials. In some plant trials the isolates resulted in significantly higher disease severity of the plants than isolates belonging to AG-2-1, AG-3 and AG-4 HGII while also having some of the highest DSIs. On the other hand, isolate 58 twice did not result in a significantly higher disease severity than even the healthy control and isolate 176 did not result in significantly higher disease severity than other isolates in plant trial 1. That being said, there was never an isolate observed that resulted in a significantly higher disease severity than any isolate belonging to AG-5-2. Interestingly, isolates of AG-5 have only been reported twice to infect faba bean (Rashid & Bernier, 1993; H. Yu et al., 2022). However, seeing as they are also able to infect similar bean crops such as Phaseolus bean and soybean (Ajayi-Oyetunde & Bradley, 2017; López-Olmos et al., 2005) and are the most common *Rhizoctonia* isolates found on legumes in Sweden as can be seen in Figure 25 (Marcou, unpublished), this may just be due to a lack of scientific research.

Finally, three isolates were tested belonging to AG-11, namely isolates 16, 20, and 101. These isolates were always found to be pathogenic towards faba bean. They were also quite aggressive, with these isolates having resulted in significantly higher disease severities than isolates belonging to AG-2-1, AG-

3 and AG-4 HGII. Isolate 16 also twice resulted the highest DSI of a plant trial, being in plant trials 1 and 3. Treatment with these isolates also never resulted in a significantly lower disease severity than any other isolate treatment. Surprisingly, AG-11 has not been reported as an AG infecting faba bean but again, they have been reported to infect similar crops like Phaseolus bean and soybean meaning this could be the result of a lack of research on the topic (Ajayi-Oyetunde & Bradley, 2017; Woodhall et al., 2020).

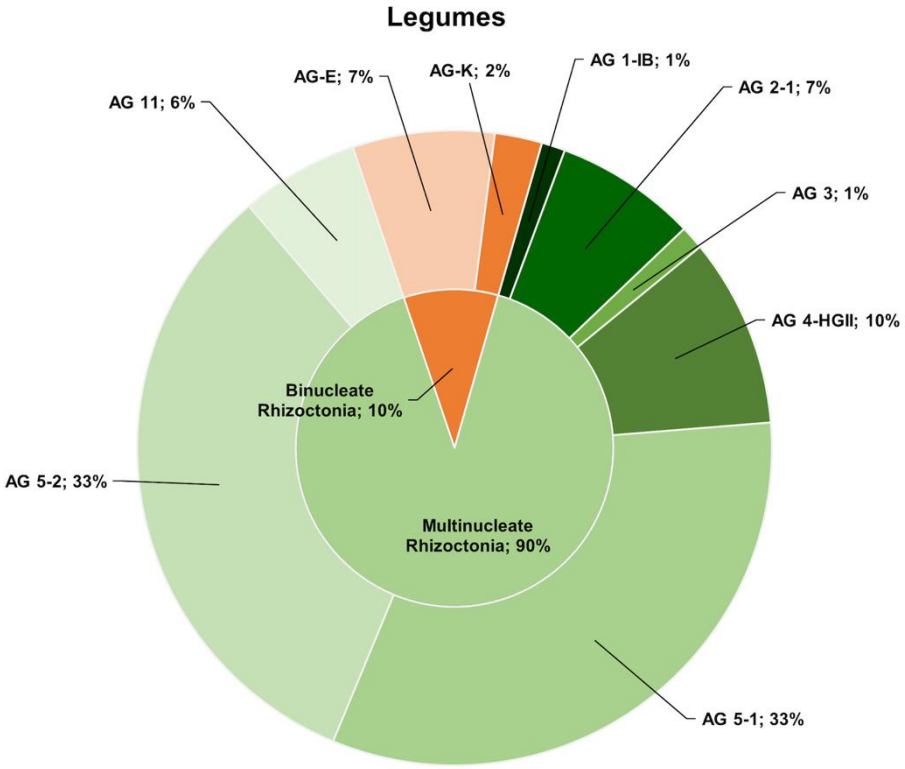


Figure 25. Distribution of the anastomosis groups (AGs) of *Rhizoctonia* isolates originating from legumes in Sweden (Marcou, unpublished).

In summary, AG-2-1 was not found to be very aggressive and AG-3 was not observed to be pathogenic Isolates from AG-5 were mostly found to be pathogenic with varying levels of aggressiveness. Excluding plant trial 1 where isolate 175 was not pathogenic, AG-4 HGII and AG-11 were consistently the most aggressive isolates on faba bean.

6.3) Effectiveness of different bacterial strains and fludioxonil for controlling *Rhizoctonia solani in vitro* and *in vivo*

6.3.1) *In vitro* antagonism of *Pseudomonas* spp. and *Bacillus* spp. against *Rhizoctonia solani*

The first experiment carried out to evaluate possible control methods for *R. solani* was *in vitro* antagonism test 1. Here, *Bacillus* strain GA1 and *Pseudomonas* strains COR58, RHF3.3-3 and UPB0736 were screened for their possible antagonistic effects against five *R. solani* isolates and AGs *in vitro*.

Seven-day old dual cultures incubated at 20 °C and 28 °C were analyzed and compared based on the PAI the bacteria caused. A summary of the statistical analyses is given in Table 26.

Table 26. Summary of the statistical data analysis of in vitro antagonism test 1. The average Percentage Area Inhibition (PAI) for each dual culture is given and rounded to the nearest whole number. Differing letters indicate that within an isolate and incubation temperature, there were statistically significant differences in PAI between bacterial strains. Colors were assigned using conditional formatting in Excel (Version 16.73 (23051401)), red indicating a low PAI and green indicating a high PAI.

	Isolate	61 (AG-3)		175 (AG-4 HGII)		139 (AG-5-1)		58 (AG-5-2)		16 (AG-11)	
Bacteria	**Temperature**	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
COR58		13 b	25 b	5 c	18 b	12 b	14	8 b	11 b	5 b	6 b
GA1		33 ab	51 a	17 b	24 b	27 a	20	18 ab	23 b	16 a	23 a
RHF3.3-3		49 a	21 b	20 ab	41 a	33 a	34	23 a	19 b	23 a	18 a
UPB0736		24 b	26 b	28 a	17 b	29 a	15	24 a	45 a	25 a	10 b

The first observation that can be made here is that COR58 does not seem to be effective in controlling *R. solani*. COR58 never resulted a significantly higher PAI than any other bacterial strain and consistently resulted in the lowest or second lowest PAI of each isolate, at 20 °C as well as at 28 °C. There also does not seem to be any scientific literature to support the antagonistic workings of COR58 against *R. solani*. Not considering COR58, the PAI values and pictures from the dual cultures seem to confirm that these bacterial strains have an inhibitory effect on the growth of *R. solani in vitro*. In the case of RHF3.3-3 and UPB0736, this has also been confirmed in previous studies (Ferrarini et al., 2022; Marahatta, 2021). GA1 seemingly has not yet been tested for its antagonistic functioning against *R. solani in vitro*. However, other *Bacillus* strains have already been shown multiple times to be capable of reducing the growth of *R. solani in vitro* (El-Mougy & Abdel-Kader, 2008; Montealegre et al., 2003; S. Singh et al., 2021).

A second observation that can be made is that there seems to be a pronounced difference in the antagonistic workings of these bacteria depending on the *R. solani* isolate and temperature. This seems especially noteworthy in dual cultures of isolates 61, 175 and 58, where bacterial strains GA1, RHF3.3-3 and UPB0736 respectively, seem to perform remarkably better at 28 °C. In each of these cases, the PAI increased around 20‰ compared to dual cultures at 20 °C, making their total PAI 40 to 50%. In the case of the dual culture of GA1 and isolate 61, this coincides with a study by Jiménez-Delgadillo et al. (2018) which found that a *B. subtilis* strain exhibited an optimal growth rate and antagonistic functioning against a *R. solani* isolate belonging to AG-3 at a temperature of 28 °C. Bacterial populations of *Pseudomonas* also seem to increase with increasing temperatures, possibly playing a role in their biocontrol activity (Mannaa & Kim, 2018). It should be noted that in stark contrast to the above, the PAI of isolate 61 caused by RHF3.3-3 more than halved when incubating at 28 °C compared to 20 °C.

As previously mentioned, because of these differences in PAI due to *R. solani* isolate and temperature, it was difficult to select optimal bacterial strains for further testing *in vivo.* GA1 was selected as it was the only strain tested belonging to *Bacillus.* Overall, RHF3.3-3 seems to work marginally better than UPB0736, as RHF3.3-3 was found to less times result in a significantly worse PAI than other bacterial strains and more times result in a significantly higher PAI than other bacterial strains compared to UPB0736. This is the reason RHF3.3-3 was selected for further testing.

Finally, while not being part of the experiment, it was noticeable that the mycelium seemed to develop pigmentation, depending on the specific *R. solani* isolate and bacterial strain. Dual cultures of isolate

61 developed a brown color, although this was also the case in the control plate. A brown color also appears in isolate 175 while isolates 139, 58 and 16 appear to develop a yellow color, although this sometimes was also the case in the control plates. This is particularly outspoken in dual cultures with GA1 as the bacterial strain. It also seems like the mycelium developed a brighter color when incubated at 20 °C as compared to 28 °C. The color development is more than likely due to the accumulation of melanin in the cell walls of hyphae, resulting in yellow and brown coloration (Duggar, 1915; Kotila, 1947; Saksena & Vaartaja, 1961; Sneh et al., 1991). Melanin can possibly be produced by *R. solani* as a reaction to the bacteria, in order to protect itself from microbial lyctic enzymes (Bloomfield & Alexander, 1967).

6.3.2) Disease control capability of *Bacillus velezensis* strain GA1, *Pseudomonas asplenii* G1 strain RHF3.3-3 and fludioxonil against *Rhizoctonia solani* inoculated *Vicia faba* L.

In plant trial 3 and 4, bacterial strains GA1 and RHF3.3-3 were evaluated for their biocontrol potential against different *R. solani* isolates in faba bean plants. These biocontrol agents were also compared to a more conventional disease control method, being seed coating with fludioxonil. Not only the disease control capabilities were evaluated, but also the germination potential of plants treated with these bacteria or fludioxonil.

Something noticeable about the experiments is that none of the disease control methods seemed to be very effective. Only once did a control method result in plants with a significantly lower disease severity than the healthy control, being the GA1 treatment of plants inoculated with isolate 175. All disease control methods carried out on isolate 61 led to an even higher amount of symptoms and DSI than the diseased controls, although there were no significant differences. Treatment with RHF3.3-3 also always resulted in a DSI higher or equal to the diseased control, plants treated with isolate 175 even having a significantly higher disease severity when treated with RHF3.3-3. Even fludioxonil did not result in significant differences compared to the healthy control. This was surprising as fludioxonil has been proven to be effective against *R. solani* on faba bean and similar crops like Phaseolus bean (Chang et al., 2014; Kataria et al., 2002). This can possibly be explained by the experimental setup. Due to seed and substrate treatments being used, the disease control evidently took place when the plants were sown. However, the plants were only inoculated seven days later. As mentioned before, this was done to attain roughly the same number of plants in each isolate treatment. Consequently, fludioxonil and the bacteria may have had lost their effectiveness. Fludioxonil could also possibly have been dispersed in the substrate, unable to protect the roots and plants.

Evaluating both experiments, GA1 does appear to be more effective in controlling *R. solani* than RHF3.3-3. While not significant as was the case for isolate 175, plants treated with isolate 139 had noticeably less disease symptoms than the diseased control. This also led to lower DSIs when plants were treated with both isolates 175 and 139, and GA1 compared to the diseased control. Similarly, El-Mougy & Abdel-Kader (2008) found *B. subtilis* capable of decreasing growth reduction of plants and symptoms of *R. solani* when applied as a bio-priming treatment on faba bean. In contrast, RHF3.3 overall resulted in more disease symptoms. This may be due to *P. asplenii* exhibiting phytopathogenic activity (Girard et al., 2020; Höfte & De Vos, 2006). While not as effective as expected, fludioxonil did result in lower DSIs for plants treated with all isolates except isolate 61.

Fludioxonil and GA1 also seemed to improve emergence of faba bean plants, even prior to being exposed to *R. solani*. *Bacillus* spp. has been found to protect plants from abiotic and biotic stress,

promoting the growth of the plants (Aydin, 2022; Radhakrishnan et al., 2017). However, in contrast to these findings, Chang et al. (2014) only found that fludioxonil could improve seed emergence in plants inoculated with *R. solani*, not those that had not been inoculated. RHF3.3-3 appeared to have the opposite effect, with significantly less plants emerging when treated with the bacteria. This can again be due to possible phytopathogenic activity by these bacteria (Girard et al., 2020; Höfte & De Vos, 2006)

6.3.3) *In vitro* effectiveness of fludioxonil against *Rhizoctonia solani*

Due to the fludioxonil treatment in the previous experiments seemingly not being very effective, an experiment was set up testing the effectiveness of fludioxonil and the fungicide Pure Celest. The EC_{50} values of fludioxonil against different *R. solani* isolates and AGs was determined and were compared with each other.

Isolate 61 of AG-3 was the most susceptible to growth inhibition by fludioxonil, having a significantly lower EC_{50} value than all other isolates, being 0.021 mg/L. A study by Djébali et al. (2014) also found that isolates from AG-3 were highly sensitive to fludioxonil, finding an even lower EC_{50} value of 0.007 mg/L. This is surprising since in the previous plant trial, fludioxonil did not seem to reduce the disease severity caused by this isolate. Most isolates however had an EC_{50} value of around 0.10 mg/L with some exceptions like isolates 175 of AG-4 HGII and isolate 62 of AG-5-1 having a significantly lower EC_{50} than most isolates and isolate 176 of AG-5-2 having a significantly higher EC_{50}. This value is slightly higher than found by Muzhinji et al. (2018), who also tested multiple AGs and found that values varied from around 0.06 to 0.09.

In the previous experiments, the seeds were coated with around 35µg per seed which is around the amount Fytoweb (2023) recommends and although slightly higher EC_{50} values were found than scientific literature suggests, this is most likely also not the reason for the subpar effectiveness of fludioxonil. It therefore seems to be the experimental setup, where inoculation took place one week after sowing and by extent the disease control, which leads to the ineffectiveness of fludioxonil in these plant trials.

6.3.4) *In vitro* antagonism of *Bacillus velezensis* strain GA1 mutants against *Rhizoctonia solani*

Since *Bacillus* strain GA1 seemed the best in controlling *R. solani in vivo*, a second *in vitro* antagonism test was carried out to evaluate which of the CLiPs GA1 produces, are the most instrumental in its antifungal activity. This was done by evaluating the PAI of different *R. solani* isolates caused by mutants of GA1 that only produce one kind of CLiP, listed in Table 4 (section 4.1.1). Again, seven-day old dual cultures incubated at 20 °C and 28 °C were used. A summary of the statistical analysis is given in Table 27.

Table 27. Summary of the statistical data analysis of in vitro antagonism test 2. The average Percentage Area Inhibition (PAI) for each dual culture is given and rounded to the nearest whole number. Differing letters indicate that within an isolate and incubation temperature, there were statistically significant differences in PAI between bacterial strains. Colors were assigned using conditional formatting in Excel (Version 16.73 (23051401)), red indicating a low PAI and green indicating a high PAI.

	Isolate	61 (AG-3)		175 (AG-4 HGII)		139 (AG-5-1)		58 (AG-5-2)		16 (AG-11)	
Bacteria	**Temperature**	20°C	28°C	20°C	28°C	20°C	20°C	28°C	20°C	28°C	20°C
GA1		70 a	85	18 a	37	17 a	36 a	28 a	47 a	20 a	36 a
GA1ΔfenA-ituA		61 b	80	4 b	31	4 b	14 b	12 b	39 bc	6 b	25 b
GA1ΔsrfaA-fenA		69 ab	91	15 a	34	16 a	31 a	28 a	40 b	21 a	34 a
GA1ΔsrfaA-ituA		73 a	83	6 b	33	7 b	17 b	15 b	34 c	9 b	27 b

As expected, wild type GA1 appears to be most effective in reducing the growth of *R. solani in vitro*, never performing significantly worse than the mutants and only twice not performing significantly better. As was the case in the previous *in vitro* antagonism test, wild type GA1 causes the highest PAI in dual cultures with isolate 61. This was also observed to be the case for the mutants and the PAI was again noticeably higher when the dual cultures were incubated at 28 °C. GA1ΔsrfaA-fenA, the mutant only capable of producing iturin, seems to perform just slightly worse than wild type GA1, only once resulting in a significantly lower PAI. The other two mutants, being GA1ΔfenA-ituA and GA1ΔsrfaA-ituA, both only capable of producing surfactin and fengycin respectively, seem to not be very effective growth inhibitors of *R. solani*, consistently resulting in significantly lower PAIs than the two other bacterial strains. Iturin, as well as surfactin and fengycin have already been found to have antifungal activity, inhibiting the *in vitro* growth of *R. solani* (Mnif et al., 2016). However, Asaka & Shoda (1996) found that while both iturin and surfactin contributed to the *in vivo* biocontrol activity of *B. subtilis* against *R. solani*, iturin likely contributed more to the biocontrol ability of the bacteria.

Looking at the pictures taken from the dual cultures, it does look like GA1ΔsrfaA-ituA, and thus fengycin, has a more profound effect on the growth of the mycelium than the surfactin producing GA1ΔfenA-ituA. The mycelium in most cases seems to grow around the GA1ΔsrfaA-ituA bacteria while this does not usually seem to be the case for GA1ΔfenA-ituA dual cultures. While only once being significant, the PAI of GA1ΔsrfaA-ituA dual cultures in most case is also slightly higher. Work by Hunter (2016) seems to confirm this suspicion, his findings being that both iturin and fengycin exhibited antifungal activity against *R. solani in vitro* while surfactin did not.

7) Conclusion

The results from this thesis support the subdivision of AG-5 of *R. solani* into two distinct subgroups based on analysis of the rDNA-ITS gene region Marcou (unpublished). A higher level of relatedness based on anastomosis reactions was observed between isolates belonging to the same proposed subgroup as relatedness between isolates belonging to the separate subgroups. Isolate 175 from AG-4 HGII was not observed to anastomose with any other AG while isolate 101 of AG-11 appeared to be somewhat related to AG-5 based on anastomosis.

Different AGs of *R. solani* appear to vary in pathogenicity and aggressiveness toward faba bean. Isolate 61 belonging to AG-3 was never shown to be pathogenic towards faba bean and isolate 40 of AG-2-1 also did not appear to be very aggressive. While results varied, isolate 175 belonging to AG-4 HGII more often than not exhibited a relatively high level of aggressiveness. Isolates from AG-5-1 consistently were pathogenic but never very aggressive while isolates from AG-5-2 again exhibited varying levels of aggressiveness from experiment to experiment. Isolates belonging to AG-11 were the only ones consistently observed to cause severe symptoms on faba bean. However, these results should be interpreted with caution since another pathogen was likely present in the substrate or seeds and symptoms affecting the emergence of the plants could not be accounted due to the experimental setup. The lower levels of aggressiveness in the last plant trial could also be the result of a suboptimal development of the inoculum.

When examining the potential of different bacterial strains for inhibiting the growth of *R. solani in vitro*, only *Pseudomonas* strain COR8 did not appear to be effective. *Pseudomonas* strains UPB0736 and RHF3.3-3, and *Bacillus* strain GA1 did substantially reduce mycelium growth. The growth inhibition did vary noticeably depending on the *R. solani* isolate and bacterial strain in the dual culture combined with the temperature at which these cultures were incubated. *R. solani* most likely developed melanin as a reaction to the bacteria.

Overall, disease control of *R. solani* on faba bean was not very effective. GA1 and fludioxonil treatment did improve the emergence of the plants and appeared to reduce disease severity to some extent. RHF3.3-3 on the other hand seems to be phytotoxic, with plants treated with the bacteria showing more symptoms and having an impaired emergence. When evaluating the *in vitro* effectiveness of fludioxinil against *R. solani*, there did not appear to be an issue with the potency of the product. This means that the ineffectiveness of fludioxonil in these plant trials is likely a consequence of the experimental setup, where fludioxonil coated seeds were sown a week prior to the inoculation with *R. solani*. This also may have impaired the effectiveness of the bacteria as biocontrol agents.

Out of the CLiPs produced by *B. velezensis* strain GA1, iturin was the most effective in reducing the growth of *R. solani in vitro*. Both surfactin and fengycin caused significantly less growth reduction in almost every dual culture tested. Of these two, fengycin did seem to be a slightly better growth inhibitor of *R. solani*. Therefore, it appears that iturin, followed by fengycin, plays the largest role in the antifungal activity of GA1.

A possible proposal for future research could be to expand the scope of this study. This can be done by including more isolates and AGs of *R. solani* or testing more bacterial strains for their biocontrol potential. For example, isolates of AG-1 and AG-7 have been shown to infect faba bean but were not evaluated here (Azimi et al., 2005). Likewise, *Pseudomonas sessilinigenes* CMR12a and *Bacillus amyloliquefaciens* have shown promising results for combatting *R. solani* (D'aes et al., 2011; Hua &

Höfte, 2015; G. Y. Yu et al., 2002). The experimental setup of the plant trials could also be altered in such a way that inoculation occurs before or simultaneously with the sowing of the plants. This way, symptoms like seed rot and pre-emergence damping-off can be accounted for and the disease control methods could possibly be more effective. Sterilizing or changing the substrate could also be an interesting adjustment to the plant trials, since there seemed to be another pathogen present in these experiments. Finally, *in vitro* testing of GA1 mutants incapable of producing one of either iturin, fengycin and surfactin would potentially yield interesting results as possible synergies between the CLiP could be evaluated.

8) References

Abawi, G. S. (1989). Root rots. In H. F. Schwartz & M. A. Pastor-Corrales (Eds.), *Bean Production Problems in the Tropics* (2nd ed., pp. 105–157).

Abbas, A., Fang, X., Iqbal, S., Naqvi, S. A. H., Mehmood, Y., Rao, M. J., Hassan, Z., Ortiz, R. M., Baazeem, A., Moustafa, M., Alrumman, S., & Negm, S. (2022). Population Genetics and Anastomosis Group's Geographical Distribution of *Rhizoctonia solani* Associated with Soybean. *Genes*, *13*(12). https://doi.org/10.3390/genes13122417

Abd-El-Khair, H., El-Dabaa, M. A., El-Nagdi, W. M., & Haggag, K. H. (2021). Using *Pseudomonas fluorescens* and Bromoxynil herbicide for controlling pests and improving growth and productivity of two faba bean cultivars. *Archives of Phytopathology and Plant Protection*, *54*(1–2), 39–55.

Abdel-Monaim, M. F. (2013). Improvement of Biocontrol of Damping-off and Root Rot/Wilt of Faba Bean by Salicylic Acid and Hydrogen Peroxide. *Mycobiology*, *41*(1), 47–55. https://doi.org/10.5941/MYCO.2013.41.1.47

Abou-Zeid, N. M., Arafa, M. K., & Sabah Attia. (2003). Biological contol of pre and post-emergence diseases on faba bean, lentil and chickpea in Egypte. *Egyptian Journal of Agricultural Research*, *81*(4), 1491–1503.

Adams, P. B. (1990). The Potential of Mycoparasites for Biological Control of Plant Diseases. *Annual Review of Phytopathology*, *28*(1), 59–72.

Agrios, G. N. (2005). *Plant pathology*. Elsevier.

Ajayi-Oyetunde, O. O., & Bradley, C. A. (2017). Identification and Characterization of *Rhizoctonia* Species Associated with Soybean Seedling Disease. *Plant Disease*, *101*(4), 520–523. https://doi.org/10.1094/PDIS-06-16-0810-RE

Ajayi-Oyetunde, O. O., & Bradley, C. A. (2018). *Rhizoctonia solani*: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. In *Plant Pathology* (Vol. 67, Issue 1, pp. 3–17). Blackwell Publishing Ltd. https://doi.org/10.1111/ppa.12733

Akladious, S. A., Gomaa, E. Z., & El-Mahdy, O. M. (2019). Efficiency of bacterial biosurfactant for biocontrol of *Rhizoctonia solani* (AG - 4) causing root rot in faba bean (*Vicia faba*) plants. *European Journal of Plant Pathology*, *153*(1), 15–35. https://doi.org/10.1007/s10658-018-01639-1

Andric, S., Meyer, T., Rigolet, A., Argüelles Arias, A., Steels, S., Hoff, G., Höfte, M., De Mot, R., McCann, A., & De Pauw, E. (2021). Chelator sensing and lipopeptide interplay mediates molecular 1 interspecies interactions between soil bacilli and pseudomonads. *BioRxiv*. https://doi.org/10.1101/2021.02.22.432387

Anitha, A., & Das, M. A. (2011). Activation of rice plant growth against *Rhizoctonia solani* using *Pseudomonas fluorescens*, *Trichoderma* and salicylic acid. *Research in Biotechnology*, *2*(4), 7–12.

Asaka, O., & Shoda, M. (1996). Biocontrol of *Rhizoctonia solani* Damping-Off of Tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology*, *62*(11), 4081–4085. https://journals.asm.org/journal/aem

Aşkin Şenocak, A., Ünal, F., & Yildirir, M. (2019). Determination of the Biocontrol Efficiency of Native *Bacillus* and Fluorescent *Pseudomonas* Isolates Against *Rhizoctonia solani* Causing Brown Patch Disease on Turfgrass Areas. *The Journal of Turkish Phytopathology*, *48*(1), 21–29.

Assunção, I. P., Nascimento, L. D., Ferreira, M. F., Oliveira, F. J., Michereff, S. J., & Lima, G. S. (2011). Reaction of faba bean genotypes to *Rhizoctonia solani* and resistance stability. *Horticultura Brasileira*, *29*, 492–497.

Aveve. (2022). *Tuinboon Scorpio*. https://www.aveve.be/nl/p020052370-tuinboon-scorpio

Aydi Ben Abdallah, R., Jabnoun-Khiareddine, H., & Daami-Remadi, M. (2019). Exploring the Beneficial Endophytic Microorganisms for Plant Growth Promotion and Crop Protection: Elucidation of Some Bioactive Secondary Metabolites Involved in Both Effects. In H. B. Singh, C. Keswani, M. S. Reddy, E. Sansinenea, & C. García-Estrada (Eds.), *Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms: Discovery and Applications* (pp. 319–352). Springer. https://doi.org/10.1007/978-981-13-5862-3_16

Aydin, M. H. (2015). Biological control of fungal plant diseases with *Trichoderma*. *Türkiye Tarımsal Araştırmalar Dergisi*, *2*(2), 135–148.

Aydin, M. H. (2022). *Rhizoctonia solani* and Its Biological Control. *Türkiye Tarımsal Araştırmalar Dergisi*, *9*(1), 118–135. https://doi.org/10.19159/tutad.1004550

Azimi, S., Farrokhi-Nejad, R., & Mousavi, J. S. (2005). *Isolation and pathogenicity of some anastomosis groups of* Rhizoctonia *associated with faba bean root and crown in Khuzestan province*. https://www.researchgate.net/publication/253233466

Baker, K. F. (1970). Types of *Rhizoctonia* Diseases and Their Occurence. In J. Parmeter (Ed.), *Rhizoctonia solani, Biology and Pathology* (pp. 125–148). University of California Press.

Baudoin, J.-P., Rocha, O., Degreef, J., Maquet, A., & Guarino, L. (2006). *Phaseolus lunatus* L. *Plant Resources of Tropical Africa (Prota)*, *1*, 141–146.

Béchet, M., Caradec, T., Hussein, W., Abderrahmani, A., Chollet, M., Leclére, V., Dubois, T., Lereclus, D., Pupin, M., & Jacques, P. (2012). Structure, biosynthesis, and properties of kurstakins, nonribosomal lipopeptides from *Bacillus* spp. *Applied Microbiology and Biotechnology*, *95*(3), 593–600. https://doi.org/10.1007/s00253-012-4181-2

Bender, C. L., Alcarón-Chaidez, F., & Gross, D. C. (1999). *Pseudomonas syringae* Phytotoxins: Mode of Action, Regulation, and Biosynthesis by Peptide and Polyketide Synthetases. *Microbiology and Molecular Biology Reviews*, *63*(2), 266–292. https://journals.asm.org/journal/mmbr

Bloomfield, B. J., & Alexander, M. (1967). Melanins and resistance of fungi to lysis. *Journal of Bacteriology*, *93*(4), 1276–1280.

Boine, B., Renner, A. C., Zellner, M., & Nechwatal, J. (2014). Quantitative methods for assessment of the impact of different crops on the inoculum density of *Rhizoctonia solani* AG2-2IIIB in soil. *European Journal of Plant Pathology*, *140*(4), 745–756. https://doi.org/10.1007/s10658-014-0506-6

Braun, A. L., & Supkoff, D. M. (1994). *Options to Methyl Bromide for the Control of Soil-Borne Diseases and Pests in California with Reference to the Netherlands*. Environmental Protection Agency, Environmental Monitoring and Pest Management Branch.

Burke, D. W., & Kraft, J. M. (1973). Responses of Beans and Peas to Root Pathogens Accumulated during Monoculture of Each Crop Species. *Phytopatholgy*, *64*(4).

Butler, E. E. (1993). *Rhizoctonia solani*. In S. Lydia & C. Kenerley (Eds.), *Biology of Sclerotial-forming Fungi* (pp. 87–112). Texas A&M University.

Butler, E. E., & Bracker, C. E. (1970). Morphology and cytology of *Rhizoctonia solani*. In J. R. Parmeter (Ed.), *Rhizoctonia solani, Biology and Pathology* (pp. 32–53). University of California Press.

Carling, D. E. (1996). Grouping in *Rhizoctonia solani* by Hyphal Anastomosis Reaction. In B. Sneh, S. Jabaji-Hare, S. Neate, & D. Gerda (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 37–48). Kluwer Academic Publishers.

Carling, D. E., Kuninaga, S., & Leiner, R. H. (1988). Relatedness within and among intraspecific groups of *Rhizoctonia solani*: A comparison of grouping by anastomosis and DNA hybridization. *Phytoparasitica*, *16*, 209–210.

Cesa-Luna, C., Geudens, N., Girard, L., De Roo, V., Maklad, H. R., Martins, J. C., Höfte, M., & De Mot, R. (2023). Charting the Lipopeptidome of Nonpathogenic *Pseudomonas*. *MSystems*, *8*(1). https://doi.org/10.1128/msystems.00988-22

Chang, K. F., Conner, R. L., Hwang, S. F., Ahmed, H. U., McLaren, D. L., Gossen, B. D., & Turnbull, G. D. (2014). Effects of seed treatments and inoculum density of *Fusarium avenaceum* and *Rhizoctonia solani* on seedling blight and root rot of faba bean. *Canadian Journal of Plant Science*, *94*(4), 693–700. https://doi.org/10.4141/CJPS2013-339

Charest, P. M. (1994). *Stachybotrys elegans*: a destructive mycoparasite of *Rhizoctonia solani*. *Mycological Research*, *98*(5), 493–505. https://doi.org/10.1016/S0953-7562(09)80467-X

Chin-A-Woeng, T. F. C., Bloemberg, G. V., & Lugtenberg, B. J. J. (2003). Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytologist*, *157*(3), 503–523. https://doi.org/10.1046/j.1469-8137.2003.00686.x

Chosdon, T., Das, K. A., Nawale, R., Kailash, B. R., Koder, S. B., & Srivastava, S. (2021). Anastomosis groups of multinucleate and binucleate *Rhizoctonia*. *Current Advances in Agricultural Sciences*, *13*(2), 71–77. https://doi.org/10.5958/2394-4471.2021.00016.2

Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J., & Moënne-Loccoz, Y. (2009). *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Letters in Applied Microbiology*, *48*(5), 505–512.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research*, *115*(3), 329–339. https://doi.org/10.1016/j.fcr.2009.09.016

D'aes, J., Hua, G. K. H., De Maeyer, K., Pannecoucque, J., Forrez, I., Ongena, M., Dietrich, L. E. P., Thomashow, L. S., Mavrodi, D. V., & Höfte, M. (2011). Biological Control of Rhizoctonia Root Rot on Bean by Phenazine- and Cyclic Lipopeptide-producing *Pseudomonas* CMR12a. *Phytopathology*, *101*(8), 996–1004. https://doi.org/10.1094/PHYTO-11-10-0315

De Candolle, A. P. (1815). *Mémoire sur les rhizoctones, noveau genre de champignons qui attaque les racines, des plantes et en particulier celle de la luzerne cultivée.* Mémoires du Museum national d'Histoire Naturelle.

De Meulemeester, M. (2015). *ZOMERVELDBOON - praktische teelthandleiding*. https://www.lcg.be/wp-content/uploads/2016/05/teeltfiche-ZOMERVELDBOON-versie.pdf

Deleu, M., Paquot, M., & Nylander, T. (2008). Effect of Fengycin, a Lipopeptide Produced by *Bacillus subtilis*, on Model Biomembranes. *Biophysical Journal*, *94*(7), 2667–2679. https://doi.org/10.1529/biophysj.107.114090

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms - A review. *Physiological and Molecular Plant Pathology*, *117*. https://doi.org/10.1016/j.pmpp.2021.101754

Djébali, N., Elkahoui, S., Taamalli, W., Hessini, K., Tarhouni, B., & Mrabet, M. (2014). Tunisian *Rhizoctonia solani* AG3 strains affect potato shoot macronutrients content, infect faba bean plants and show in vitro resistance to azoxystrobin. *Australasian Plant Pathology*, *43*(3), 347–358. https://doi.org/10.1007/s13313-014-0277-8

Djonović, S., Vittone, G., Mendoza-Herrera, A., & Kenerley, C. M. (2007). Enhanced biocontrol activity of *Trichoderma virens* transformants constitutively coexpressing β-1,3- and β-1,6-glucanase genes. *Molecular Plant Pathology*, *8*(4), 469–480. https://doi.org/10.1111/j.1364-3703.2007.00407.x

Duggar, B. M. (1915). *Rhizoctonia Crocorum (Pers.) DC. and R. Solani Kühn (Corticium Vagum B. & C.), with Notes on Other Species*. *2*(3), 403–458. https://about.jstor.org/terms

El-Dabaa, M. A. T., Abd-El-Khair, H., & El-Nagdi, W. M. A. (2019). Field application of Clethodim herbicide combined with *Trichoderma* spp. For controlling weeds, root knot nematodes and *Rhizoctonia* root rot disease in two faba bean cultivars. *Journal of Plant Protection Research*, *59*(2), 255–264. https://doi.org/10.24425/jppr.2019.129287

El-Mougy, N. S., & Abdel-Kader, M. M. (2008). Long-term activity of bio-priming seed treatment for biological control of faba bean root rot pathogens. *Australasian Plant Pathology*, *37*(5), 464–471. https://doi.org/10.1071/AP08043

Engelkes, C. A., & Windels, C. E. (1996). Susceptibility of sugar beet and beans to *Rhizoctonia solani* AG-2-2 IIIB and AG-2-2 IV. *Plant Disease (USA)*, *80*(12), 1413–1417.

EUR-Lex. (2021). *Substances depleting the ozone layer*. https://eur-lex.europa.eu/EN/legal-content/summary/substances-depleting-the-ozone-layer.html#:~:text=Use%20of%20methyl%20bromide%20has,be%20used%20in%20certain%20circumstances.

Falardeau, J., Wise, C., Novitsky, L., & Avis, T. J. (2013). Ecological and Mechanistic Insights Into the Direct and Indirect Antimicrobial Properties of *Bacillus subtilis* Lipopeptides on Plant Pathogens. *Journal of Chemical Ecology*, *39*(7), 869–878. https://doi.org/10.1007/s10886-013-0319-7

Fan, B., Blom, J., Klenk, H. P., & Borriss, R. (2017). *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an "Operational Group *B. amyloliquefaciens*" within the *B. subtilis* species complex. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00022

FAOSTAT. (2023). *FAOSTAT: Crops and livestock products*. https://www.fao.org/faostat/en/#data/QCL

Ferrarini, E., Špacapan, M., Lam, V. B., McCann, A., Cesa-Luna, C., Marahatta, B. P., De Pauw, E., De Mot, R., Venturi, V., & Höfte, M. (2022). Versatile role of *Pseudomonas fuscovaginae* cyclic lipopeptides in plant and microbial interactions. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.1008980

Fira, D., Dimkić, I., Berić, T., Lozo, J., & Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, *285*, 44–55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Fytoweb. (2023). *Fytoweb: Gewasbeschermingsmiddelen en Bemestingsproducten*. https://apps.health.belgium.be/fytoweb/pages/public/index.xhtml;jsessionid=kbg4ineozBuxxL1fdYSBwrOmBwzSIQisPJ6KF8oB.node5?lang=nl&dswid=-433

Geudens, N., & Martins, J. C. (2018). Cyclic lipodepsipeptides from *Pseudomonas* spp. - Biological Swiss-Army knives. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01867

Girard, L., Höfte, M., & Mot, R. De. (2020). Lipopeptide families at the interface between pathogenic and beneficial *Pseudomonas*-plant interactions. *Critical Reviews in Microbiology*, *46*(4), 397–419. https://doi.org/10.1080/1040841X.2020.1794790

Girard, L., Lood, C., Höfte, M., Vandamme, P., Rokni-Zadeh, H., van Noort, V., Lavigne, R., & De Mot, R. (2021). The Ever-Expanding *Pseudomonas* genus: Description of 43 New Species and Partition of the *Pseudomonas putida* Group. *Microorganisms*, *9*(8). https://doi.org/10.3390/microorganisms9081766

Gondal, A. S., Rauf, A., & Naz, F. (2019). Anastomosis Groups of *Rhizoctonia solani* associated with tomato foot rot in Pothohar Region of Pakistan. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-40043-5

González García, V., Portal Onco, M. A., & Rubio Susan, V. (2006). Review. Biology and Systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research*, *4*(1), 55–79.

Gross, H., & Loper, J. E. (2009). Genomics of secondary metabolite production by *Pseudomonas* spp. *Natural Product Reports*, *26*(11), 1408–1446.

Harveson, R. M., Smith, J. A., & Stroup, W. W. (2005). Improving Root Health and Yield of Dry Beans in the Nebraska Panhandle with a New Technique for Reducing Soil Compaction. *Plant Disease*, *89*(3), 279–284. https://doi.org/10.1094/PD-89-0279

Hassan, G. M., El-Feky, Z. A., Hemada, N. F., & Sayed, M. A. (2015). Isolation and molecular characterization of Egyptian *Trichoderma* and assessment of their antagonistic potential against *Rhizoctonia solani*. *Journal of Microbiology, Biotechnology and Food Sciences*, *4*(6), 495–502. https://doi.org/10.15414/jmbfs.2015.4.6.495-502

Hassanein, A. M., El-Garhy, A. M., & Mekhemar, G. A. A. (2006). Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root-rot. *Journal of Agricultural Chemistry and Biotechnology*, *31*(2), 963–980.

Herr, L. J. (1993). Host sources, virulence and overwinter survival of *Rhizoctonia solani* anastomosis groups isolated from field lettuce with bottom rot symptoms. *Crop Protection*, *12*(7), 521–526.

Höfte, M. (2021). *Herkenning en diagnose van plantenziekten, plagen en onkruiden: Deel plantenziekten - slides*. Ghent University - Departement of Plants and Crops.

Höfte, M., & De Vos, P. (2006). Plant pathogenic *Pseudomonas* species. *Plant-Associated Bacteria*, 507–533.

Howell, C. R., Stipanovic, R. D., & Lumsden, R. D. (1993). Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. *Biocontrol Science and Technology*, *3*(4), 435–441.

Hua, G. K. H. (2014). *Integrated Control of Rhizoctonia Diseases on Bean and Cabbage*. (Doctoral dissertation, Ghent University).

Hua, G. K. H., & Höfte, M. (2015). The involvement of phenazines and cyclic lipopeptide sessilin in biocontrol of Rhizoctonia root rot on bean (*Phaseolus vulgaris*) by *Pseudomonas* sp. CMR12a is influenced by substrate composition. *Plant and Soil*, *388*, 243–253. https://doi.org/10.1007/s11104-014-2327-y

Huang, X., Zhang, N., Yong, X., Yang, X., & Shen, Q. (2012). Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. *Microbiological Research*, *167*(3), 135–143. https://doi.org/10.1016/j.micres.2011.06.002

Huber, D. M., & Sumner, D. R. (1996). Suppressive Soil Amendments for the Control of *Rhizoctonia* Species. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 433–443). Kluwer Academic Publishers.

Hunter, C. H. (2016). *Screening of aerobic endospore-forming bacterial isolates as candidate biocontrol agents against Rhizoctonia solani*. (Doctoral dissertation).

Infantino, A., Kharrat, M., Riccioni, L., Coyne, C. J., McPhee, K. E., & Grünwald, N. J. (2006). Screening techniques and sources of resistance to root diseases in cool season food legumes. *Euphytica*, *147*, 201–221. https://doi.org/10.1007/s10681-006-6963-z

Jaaffar, A. K. M., Parejko, J. A., Paulitz, T. C., Weller, D. M., & Thomashow, L. S. (2017). Sensitivity of *Rhizoctonia* Isolates to Phenazine-1-Carboxylic Acid and Biological Control by Phenazine-Producing *Pseudomonas* spp. *Phytopathology*, *107*(6), 692–703. https://doi.org/10.1094/PHYTO-07-16-0257-R

Jacob, C., Jamier, V., & Ba, L. A. (2011). Redox active secondary metabolites. *Current Opinion in Chemical Biology*, *15*(1), 149–155. https://doi.org/10.1016/j.cbpa.2010.10.015

Jiménez-Delgadillo, R., Valdés-Rodríguez, S. E., Olalde-Portugal, V., Abraham-Juárez, R., & García-Hernández, J. L. (2018). Effect of pH and temperature on the growth and antagonistic activity of *Bacillus subtilis* on *Rhizoctonia solani*. *Revista Mexicana de Fitopatología, Mexican Journal of Phytopathology*, *36*(2), 256–275. https://doi.org/10.18781/R.MEX.FIT.1711-3

Katan, J. (1996). Soil Solarization for the Control of Diseases Caused by *Rhizoctonia* Spp. In B. Sneh, Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 423–432). Kluwer Academic Publishers.

Kataria, H. R., & Gisi, U. (1996). Chemical Control of *Rhizoctonia* Species. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 537–547). Kluwer Academic Publishers.

Kataria, H. R., Wilmsmeier, B., & Buchenauer, H. (2002). Efficacy of *Pseudomonas fluorescens* strains and some modern fungicides for control of *Rhizoctonia solani* AG-4 in bean and cucumber. *Zeitschrift Für Pflanzenkrankheiten Und Pflanzenschutz, Journal of Plant Diseases and Protection*, *109*(4), 384–400. https://about.jstor.org/terms

Keijer, J. (1996). The Initial Steps of the Infection Process in *Rhizoctonia solani*. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Patholkogy and Disease Control* (pp. 149–162). Kluwer Academic Publishers.

Kirk, W. D. J. (2004). Faba bean: *Vicia faba*. *Bee World*, *85*(3), 60–62. https://doi.org/10.1080/0005772X.2004.11099625

Kotila, J. E. (1947). Rhizoctonia foliage blight of sugar beets. *Journal of Agricultural Research*, *74*(11), 289–314.

Kumari, S. G., & Makkouk, K. M. (2007). Virus Diseases of Faba Bean (*Vicia faba* L.) in Asia and Africa. *Plant Viruses*, *1*(1), 93–105.

Kuninaga, S., Carling, D. E., Takeuchi, T., & Yokosawa, R. (2000). Comparison of rDNA-ITS Sequences between Potato and Tobacco Strains in *Rhizoctonia solani* AG-3. *Journal of General Plant Pathology*, *66*, 2–11.

Lam Bach, V. (2021). *Pseudomonas and Bacillus spp. associated with rice grown in acid sulfate soils in Vietnam: taxonomy and potential for biocontrol and biostimulation*. (Doctoral dissertation, Ghent University).

Laursen, J. B., & Nielsen, J. (2004). Phenazine Natural Products: Biosynthesis, Synthetic Analogues, and Biological Activity. *Chemical Reviews*, *104*(3), 1663–1685. https://doi.org/10.1021/cr020473j

Leach, L. D., & Garber, R. H. (1970). Control of *Rhizoctonia*. In J. R. Parmeter (Ed.), *Rhizoctonia solani: biology and pathology* (pp. 189–199). University of California Press.

Li, C., Guo, Z., Zhou, S., Han, Q., Zhang, M., Peng, Y., Hsiang, T., & Chen, X. (2021). Evolutionary and genomic comparisons of hybrid uninucleate and nonhybrid *Rhizoctonia* fungi. *Communications Biology*, *4*(1). https://doi.org/10.1038/s42003-021-01724-y

López-Olmos, K., Hernández-Delgado, S., & Mayek-Pérez, N. (2005). AFLP Fingerprinting for Identification of Anastomosis Groups of *Rhizoctonia solani* Kühn from Common Bean (*Phaseolus vulgaris* L.) in México. *Revista Mexicana de Fitopatología*, *23*(2), 147–151. http://www.redalyc.org/articulo.oa?id=61223206

Lübeck, M. (2004). Molecular characterization of *Rhizoctonia solani*. In D. K. Arora & G. G. Khachatourians (Eds.), *Applied mycology and biotechnology* (Fungal Genomics, Vol. 4, pp. 205–224). Elsevier.

Lucas, J. A., Hawkins, N. J., & Fraaije, B. A. (2015). The Evolution of Fungicide Resistance. *Advances in Applied Microbiology*, *90*, 29–92. https://doi.org/10.1016/bs.aambs.2014.09.001

Ma, Z., Hoang Hua, G. K. H., Ongena, M., & Höfte, M. (2016). Role of phenazines and cyclic lipopeptides produced by *pseudomonas* sp. CMR12a in induced systemic resistance on rice and bean. *Environmental Microbiology Reports*, *8*(5), 896–904. https://doi.org/10.1111/1758-2229.12454

Maalouf, F., Nawar, M., Hamwieh, A., Amri, A., Xuxiao, Z., Shiying, B., & Tao, Y. (2013). Faba bean genetics and genomics and their use in breeding program. In M. Singh, H. D. UpadHyaya, & I. Singh Bisht (Eds.), *Genetic and genomic resources for grain legume improvement* (pp. 113–136). Elsevier.

Mahmoud, Y. A., Gaafar, R. M., & Mubarak, H. M. (2007). Genetic Diversity among Nile Delta Isolates of *Rhizoctonia solani* Kühn Based on Pathogenicity, Compatibility, Isozyme Analysis and Total Protein Pattern. *Turkish Journal of Botany*, *31*(1), 19–29. https://journals.tubitak.gov.tr/botany/vol31/iss1/3

Malviya, D., Sahu, P. K., Singh, U. B., Paul, S., Gupta, A., Gupta, A. R., Singh, S., Kumar, M., Paul, D., Rai, J. P., Singh, H. V., & Brahmaprakash, G. P. (2020). Lesson from ecotoxicity: Revisiting the microbial lipopeptides for the management of emerging diseases for crop protection. *International Journal of Environmental Research and Public Health*, *17*(4). https://doi.org/10.3390/ijerph17041434

Mannaa, M., & Kim, K. D. (2018). Effect of Temperature and Relative Humidity on Growth of *Aspergillus* and *Penicillium* spp. and Biocontrol Activity of *Pseudomonas protegens* AS15 against Aflatoxigenic *Aspergillus flavus* in Stored Rice Grains. *Mycobiology*, *46*(3), 287–295. https://doi.org/10.1080/12298093.2018.1505247

Manning, W. J., Crossan, D. F., & Morton, D. J. (1967). Effect of planting depth and asphalt mulch on Rhizoctonia root and hypocotyl rot of snapbean. *Plant Disease Reporter*, *51*(3), 158–160.

Marahatta, B. P. (2021). *Biocontrol Potential of Beneficial and Plant Pathogenic strains related to Pseudomonas fuscovaginae (Pseudomonadales, Pseudomonadacea)*. Ghent University.

Marcou, S., Wikström, M., Ragnarsson, S., Persson, L., & Höfte, M. (2021). Occurrence and Anastomosis Grouping of *Rhizoctonia* spp. Inducing Black Scurf and Greyish-White Felt-Like Mycelium on Carrot in Sweden. *Journal of Fungi*, *7*(5). https://doi.org/10.3390/jof7050396

Mavrodi, D. V., Blankenfeldt, W., & Thomashow, L. S. (2006). Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. *Annual Review of Phytopathology*, *44*, 417–445. https://doi.org/10.1146/annurev.phyto.44.013106.145710

Misra, J., Merca, S., & Mew, T. (1994). Fungal pathogens. In T. Mew & J. Misra (Eds.), *Manual of rice seed health testing* (pp. 75–89). IRRI.

Mnif, I., Grau-Campistany, A., Coronel-León, J., Hammami, I., Triki, M. A., Manresa, A., & Ghribi, D. (2016). Purification and identification of *Bacillus subtilis* SPB1 lipopeptide biosurfactant exhibiting antifungal activity against *Rhizoctonia bataticola* and *Rhizoctonia solani*. *Environmental Science and Pollution Research*, *23*(7), 6690–6699. https://doi.org/10.1007/s11356-015-5826-3

Mohamed, M. H., Gado, E. A. M., El-Deeb, S. H., & Mostafa, M. H. (2014a). Behavior of fungus *Rhizoctonia solani* under faba bean cotyledons when away from host and the effect of its starvation on aggressiveness. *Journal of Yeast and Fungal Research*, *5*(1), 1–8. https://doi.org/10.5897/jyfr2013.0129

Mohamed, M. H., Gado, E., El-Deeb, S., & Mostafa, M. (2014b). Effect of nitrate levels as a fertilizer or as a fungal nutrition on the aggressiveness of *Rhizoctonia solani* on faba bean. *European Journal of Advanced Research in Biological and Life Sciences*, *2*(2). https://www.researchgate.net/publication/273948290

Mohamed, M. H., Gado, E., El-Deeb Samir, & Mostafa, H. M. (2015). Obstruction of Signal Transduction between Faba Bean and *Rhizoctonia solani* during Pathogenesis and its Impact on Root Rot Disease. *International Scientific Research Journal*, *1*, 99–107. https://doi.org/10.18483/irjsci.50

Montealegre, J. R., Reyes, R., María Pérez, L., Herrera, R., Silva, P., & Besoain, X. (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. *Electronic Journal of Biotechnology*, *6*(2), 115–127. http://www.ejbiotechnology.info/content/vol6/issue2/full/8

Mori, M., Bannai, T., & Misawa, T. (2021). First report of leaf blight and petiole rot of carrot caused by *Rhizoctonia solani* AG-1 IB. *Journal of General Plant Pathology*, *87*, 42–45. https://doi.org/10.1007/s10327-020-00966-8

Mueller, T. (2021). *Rhizoctonia damping-off, blight and rot (Rhizoctonia solani)*. https://www.forestryimages.org/browse/detail.cfm?imgnum=5604688

Muzhinji, N., Woodhall, J. W., Truter, M., & Van Der Waals, J. E. (2018). Variation in Fungicide Sensitivity Among *Rhizoctonia* Isolates Recovered from Potatoes in South Africa. *Plant Disease*, *102*(8), 1520–1526. https://doi.org/10.1094/PDIS-09-17-1470-RE

Mwiindilila, C. N. (1984). *Characterisation of Rhizoctonia solani (Thanatephorus cucumeris) isolates in Manitoba*. The University Of Manitoba.

Nagaraj, B. T., Gururaj, S., Pramesh, D., Naik, M. K., Paril Mahanthesh B, Yadav, M. K., & Patil, N. B. (2019). Morphological, genetic and virulence diversity of *Rhizoctonia solani* isolates from different rice growing regions of Southern India. *Research Journal of Biotechnology*, *14*(5), 16–23. https://www.researchgate.net/publication/333113880

Naito, S. (1996). Basidiospore Dispersal and Survival. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 197–205). Kluwer Academic Publishers.

Nakkeeran, S., Vinodkumar, S., Senthilraya, C., & Renukadevi, P. (2019). Antimicrobial peptides of *Bacillus* species: Biosynthesis, mode of action and their role in plant disease management. In R. N. Pandey, B. N. Chakraborty, D. Singh, & S. Pratibha (Eds.), *Microbial Antagonists: Their Role in Biological Control of Plant Diseases* (pp. 487–514). Today & Tomorrow's Printers and Publishers. https://www.researchgate.net/publication/335169272

National Library of Medicine. (n.d.). *PubChem*. Retrieved April 11, 2023, from https://pubchem.ncbi.nlm.nih.gov/

Neuvel, J. (1991). *Teelt van TUINBONEN*. https://edepot.wur.nl/253088

Ongena, M., & Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends in Microbiology*, *16*(3), 115–125. https://doi.org/10.1016/j.tim.2007.12.009

Oni, F. E., Geudens, N., Omoboye, O. O., Bertier, L., Hua, H. G. K., Adiobo, A., Sinnaeve, D., Martins, J. C., & Höfte, M. (2019). Fluorescent *Pseudomonas* and cyclic lipopeptide diversity in the

rhizosphere of cocoyam (*Xanthosoma sagittifolium*). *Environmental Microbiology*, *21*(3), 1019–1034. https://doi.org/10.1111/1462-2920.14520

Oni, F. E., Geudens, N., Onyeka, J. T., Olorunleke, O. F., Salami, A. E., Omoboye, O. O., Arias, A. A., Adiobo, A., De Neve, S., Ongena, M., Martins, J. C., & Höfte, M. (2020). Cyclic lipopeptide-producing *Pseudomonas koreensis* group strains dominate the cocoyam rhizosphere of a *Pythium* root rot suppressive soil contrasting with *P. putida* prominence in conducive soils. *Environmental Microbiology*, *22*(12), 5137–5155. https://doi.org/10.1111/1462-2920.15127

Panella, L., & Ruppel, E. G. (1996). Availability of germplasm for resistance against *Rhizoctonia* spp. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 515–527). Kluwer Academic Publishers.

Papavizas G. C. (1970). Colonization and growth of *Rhizoctonia solani* in soil. In J. Parmeter (Ed.), *Rhizoctonia solani, Biology and Pathology* (pp. 108–122). Univeristy of California Press.

Papavizas, G. C., & Davey, C. B. (1962). Activity of *Rhizoctonia* in soil as affected by carbon dioxide. *Phytopathology (EUA)*, *52*(8), 759–766.

Papavizas, G. C., & Lewis, J. A. (1979). Integrated control of *Rhizoctonia solani*. In B. Schippers & W. Gaens (Eds.), *Soil-borne plant pathogens* (pp. 415–424). Academic Press.

Park, M. G., Choi, J., Hong, Y. S., Park, C. G., Kim, B. G., Lee, S. Y., Lim, H. J., Mo, H. H., Lim, E., & Cha, W. (2020). Negative effect of methyl bromide fumigation work on the central nervous system. *PLoS ONE*, *15*(8). https://doi.org/10.1371/journal.pone.0236694

Parmeter, J. R., & Whitney, H. S. (1970). Taxonomy and nomenclature of the imperfect state. In J. R. Parmeter (Ed.), *Rhizoctonia solani, Biology and Pathology* (pp. 7–19). University of California Press.

Patel, H. K., da Silva, D. P., Devescovi, G., Maraite, H., Paszkiewicz, K., Studholme, D. J., & Venturia, V. (2012). Draft Genome Sequence of *Pseudomonas fuscovaginae*, a Broad-Host-Range Pathogen of Plants. *Journal of Bacteriology*, *194*(10), 2765–2766. https://doi.org/10.1128/JB.00341-12

Paul, S. K., Gupta, D. R., Mahmud, N. U., Muzahid, A. N. M., & Islam, M. T. (2022). First Report of Collar and Root Rot of Faba Bean Caused by *Rhizoctonia solani* AG-2-2 IIIB in Bangladesh. *Plant Disease*, *106*(3). https://doi.org/10.1094/PDIS-08-21-1603-PDN

Prabhu, S., & Rajeswari, D. (2018). Nutritional and Biological properties of *Vicia faba* L.: A perspective review. *International Food Research Journal*, *25*(4), 1332–1340.

Raaijmakers, J. M., De Bruijn, I., & De Kock, M. J. D. (2006). Cyclic Lipopeptide Production by Plant-Associated *Pseudomonas* spp.: Diversity, Activity, Biosynthesis, and Regulation. *Molecular Plant-Microbe Interactions*, *19*(7), 699–710. https://doi.org/10.1094/MPMI

Raaijmakers, J. M., de Bruijn, I., Nybroe, O., & Ongena, M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS Microbiology Reviews*, *34*(6), 1037–1062. https://doi.org/10.1111/j.1574-6976.2010.00221.x

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*. https://doi.org/10.3389/fphys.2017.00667

Rashid, K. Y., & Bernier, C. C. (1993). Genetic diversity among isolates of *Rhizoctonia solani* and sources of resistance in *Vicia faba*. *Canadian Journal of Plant Pathology*, *15*(1), 23–28.

Rickerl, D. H., Curl, E. A., & Touchton, J. T. (1989). Tillage and rotation effects on Collembola populations and *Rhizoctonia* infestation. *Soil & Tillage Research*, *15*(1–2), 41–49.

Ron, E. Z., & Rosenberg, E. (2001). Natural roles of biosurfactants. *Environmental Microbiology*, *3*(4), 229–236. https://doi.org/10.1046/j.1462-2920.2001.00190.x

Saikia, R., Kumar, R., Arora, D. K., Gogoi, D. K., & Azad, P. (2006). *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: Production of salicylic acid and Peroxidases. *Folia Microbiologica*, *51*(5), 375–380. http://www.biomed.cas.cz/mbu/folia/

Saksena, H. K., & Vaartaja, O. (1961). Taxonomy, morphology and pathogenicity of Rhizoctonia species from forest nurseries. *Canadian Journal of Botany*, *39*(3), 627–647.

Salt, G. A. (1982). Factors Affecting Resistance to Root Rot and Wilt Diseases. In G. Hawtin & C. Webb (Eds.), *Faba Bean Improvement* (pp. 259–270). Springer Netherlands. https://doi.org/10.1007/978-94-009-7499-9_27

Sande, D., Mullen, J., Wetzstein, M., & Houston, J. (2011). Environmental Impacts from Pesticide Use: A Case Study of Soil Fumigation in Florida Tomato Production. *International Journal of Environmental Research and Public Health*, *8*(12), 4649–4661. https://doi.org/10.3390/ijerph8124649

Schneider, J. H. M., Salazar, O., Rubio, V., & Keijer, J. (1997). Identification of *Rhizoctonia solani* associated with field-grown tulips using ITS rDNA polymorphism and pectic zymograms. *European Journal of Plant Pathology*, *103*, 607–622.

Schwartz, H. F. (2008). *Rhizoctonia damping-off, blight and rot (Rhizoctonia solani)*. https://www.forestryimages.org/browse/detail.cfm?imgnum=5361371

Seydlová, G., Čabala, R., & Svobodová, J. (2011). Surfactin - Novel Solutions for Global Issues. In M. A. Komorowska & S. Olstyńska-Janus (Eds.), *Biomedical Engineering, Trends, Research and Technologies* (Vol. 13, pp. 305–330). InTech.

Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A., & Maillard, M. N. (2021). Fava bean (*Vicia faba* L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. *Comprehensive Reviews in Food Science and Food Safety*, *20*(1), 401–428. https://doi.org/10.1111/1541-4337.12687

Sharma, D. (2004). *Plant Pathology*. Rastogi Publications.

Sharon, M., Kuninaga, S., Hyakumachi, M., & Sneh, B. (2006). The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience*, *47*(6), 299–316. https://doi.org/10.1007/s10267-006-0320-x

Singh, A. K., Bharati, R. C., Manibhushan, N. C., & Pedpati, A. (2013). An assessment of faba bean (*Vicia faba* L.) current status and future prospect. *African Journal of Agricultural Research*, *8*(50), 6634–6641. https://doi.org/10.5897/AJAR2013.7335

Singh, S., Balodi, R., Meena, P. N., & Singhal, S. (2021). Biocontrol activity of *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita*, *Fusarium oxysporum* and *Rhizoctonia solani*. *Indian Phytopathology*, *74*(3), 703–714. https://doi.org/10.1007/s42360-021-00368-6

Sneh, B., Burpee, L., & Ogoshi, A. (1991). *Identification of Rhizoctonia Species*. The American Phytopathological Society.

Sreejith, S., Aswani, R., & Radhakrishnan, E. K. (2019). Agriculturally Important Biosynthetic Features of Endophytic Microorganisms. In *Seed Endophytes: Biology and Biotechnology* (pp. 423–447). Springer International Publishing. https://doi.org/10.1007/978-3-030-10504-4_20

Stalpers, J. A., & Anderson, T. F. (1996). A Synopsis of the Taxonomy of Teleomorphs Connected with *Rhizoctonia* S.L. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 49–63). Kluwer Academic Publishers.

Syngenta. (2023). *Celest*. https://www.syngenta.be/ons-product/seedcare/akkerbouw/producten/celest

Touré, Y., Ongena, M., Jacques, P., Guiro, A., & Thonart, P. (2004). Role of lipopeptides produced by Bacillus subtilits GA1 in the reduction of grey mould disease caused by Botrytis cinerea on apple. *Journal of Applied Microbiology*, *96*(5), 1151–1160.

Tran, H., Ficke, A., Asiimwe, T., Höfte, M., & Raaijmakers, J. M. (2007). Role of the cyclic lipopeptide massetolide a in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytologist*, *175*(4), 731–742. https://doi.org/10.1111/j.1469-8137.2007.02138.x

Tu, C.-C., Hsieh, T.-F., & Chang, Y.-C. (1996). Vegetable Diseases Incited by *Rhizoctonia* Spp. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 369–377). Kluwer Academic Publishers.

Tu, J. C., & Tan, C. S. (1991). Effect of soil compaction on growth, yield and root rots of white beans in clay loam and sandy loam soil. *Soil Biology and Biochemistry*, *23*(3), 233–238.

Van Bruggen, A. H., Grünwald, N. J., & Bolda, M. (1996). Cultural Methods and Soil Nutrient Status in Low and High Input Agricultural Systems, as They Affect *Rhizoctonia* Species. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 407–421). Kluwer Academic Publishers.

Van den Boogert, P. H. J. F. (1996). Mycoparasitism and Biocontrol. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 485–493). Kluwer Academic Publishers.

Van den Broeck, I. (2017). *WINTERVELDBOON praktische teelthandleiding*. https://inagro.be/sites/default/files/media/files/2021-07/Teeltfiche_winterveldboon.pdf

Weinhold, A. R., & Sinclair, J. B. (1996). *Rhizoctonia solani*: Penetration, Colonization, and Host Response. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 163–174). Kluwer Academic Publishers.

Weller, D. M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*, *26*(1), 379–407.

Woodhall, J. W., Brown, L., Harrington, M., Pizolotto, C. A., Keith, S., & Marshall, J. M. (2020). First Report of *Rhizoctonia solani* AG 11 Causing Stem Rot of *Phaseolus vulgaris* in Idaho. *Plant Disease*, *104*(9), 2516–2516. https://doi.org/10.1094/PDIS-03-20-0666-PDN

Yang, J., Verma, P. R., & Tewari, J. P. (1992). Histopathology of resistant mustard and susceptible canola hypocotyls infected by *Rhizoctonia solani*. *Mycological Research*, *96*(3), 171–179. https://doi.org/10.1016/S0953-7562(09)80962-3

Yin, C., Hulbert, S. H., Schroeder, Kurtis. L., Mavrodi, O., Mavrodi, D., Dhingra, A., Schillinger, W. F., & Paulitz, T. C. (2013). Role of bacterial communities in the natural suppression of *Rhizoctonia solani* bare patch disease of wheat (*Triticum aestivum* L.). *Applied and Environmental Microbiology*, *79*(23), 7428–7438.

Yu, G. Y., Sinclair, J. B., Hartman, G. L., & Bertagnolli, B. L. (2002). Production of iturin A by Bacillus amyloliquefaciens suppressing Rhizoctonia solani. *Soil Biology and Biochemistry*, *34*(7), 955–963. www.elsevier.com/locate/soilbio

Yu, H., Zhou, Q., Hwang, S. F., Ho, A. J., Chang, K. F., Strelkov, S. E., He, Y., Conner, R. L., & Harding, M. W. (2022). Pathogenicity, anastomosis groups, host range, and genetic diversity of *Rhizoctonia* species isolated from soybean, pea, and other crops in Alberta and Manitoba, Canada. *Canadian Journal of Plant Science*, *102*(2), 301–315. https://doi.org/10.1139/cjps-2021-0039

9) Appendix

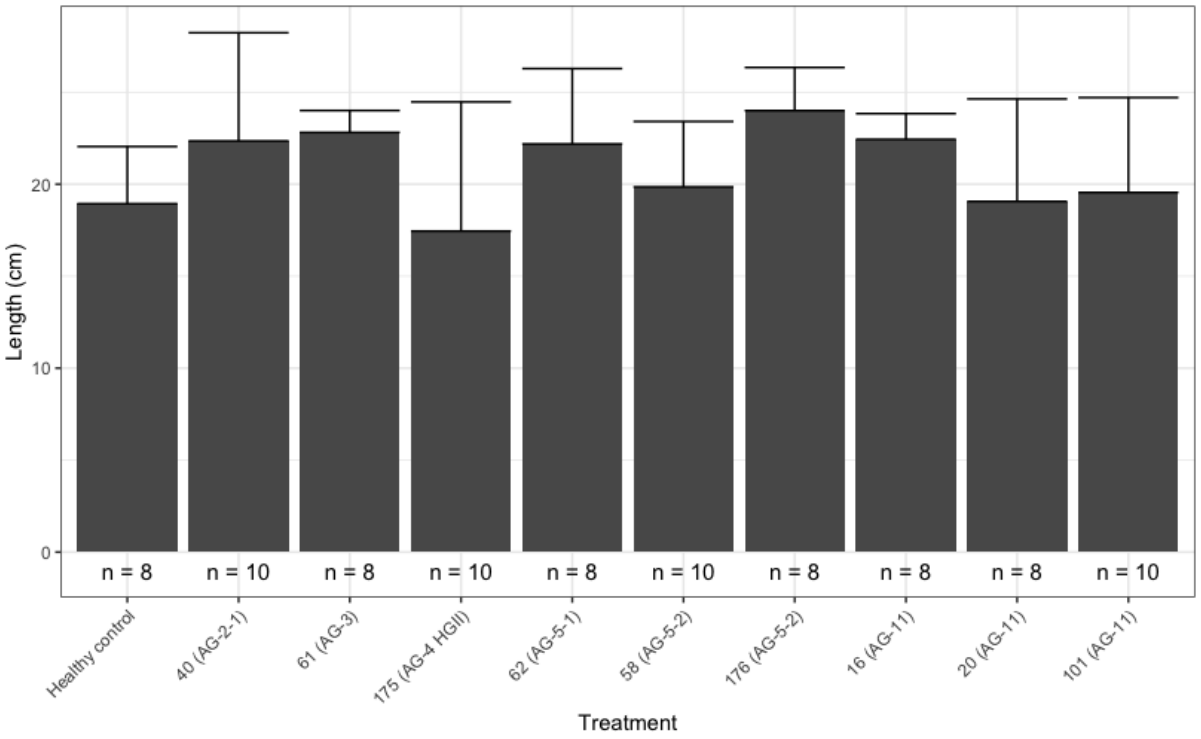


Figure A 1. The average length of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 2. The error bars indicate the standard deviations on the measurements. There were no statistically significant differences found between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

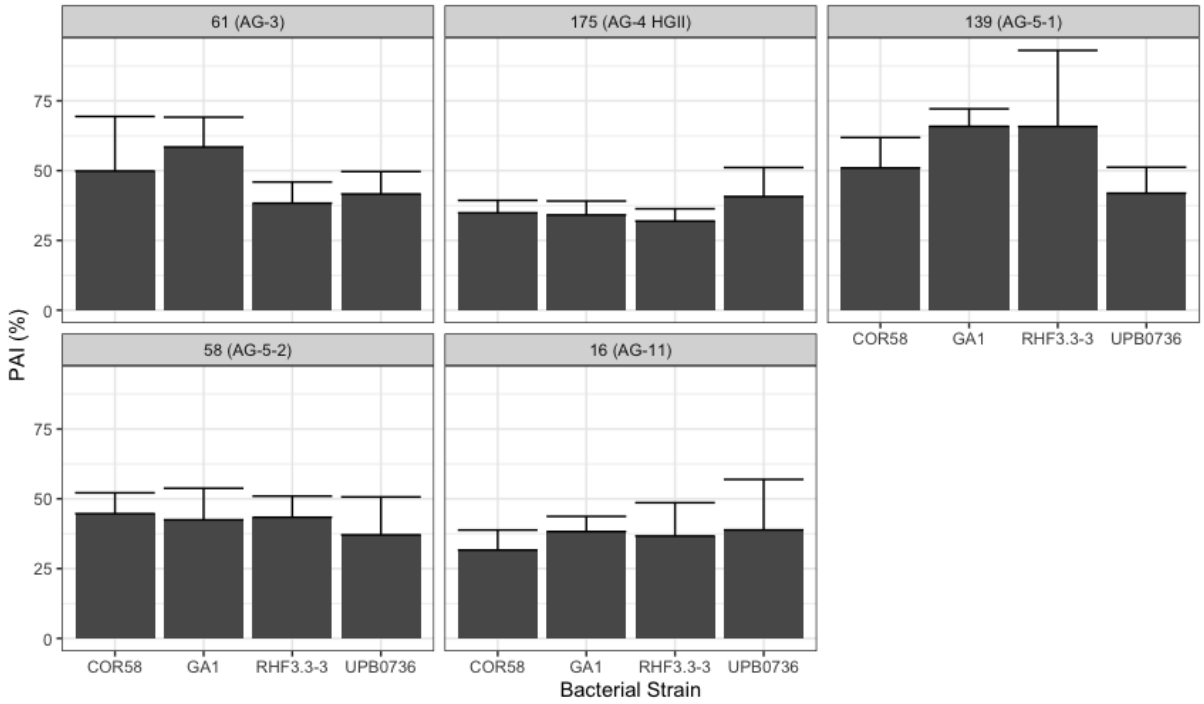
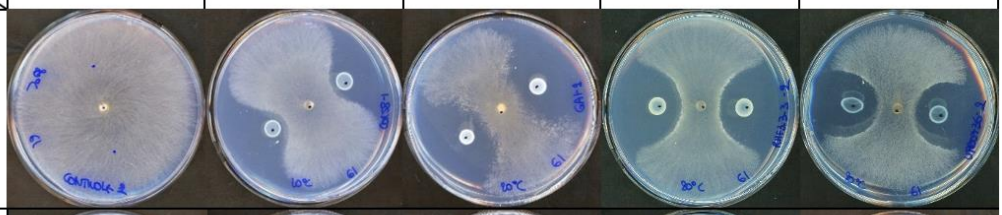
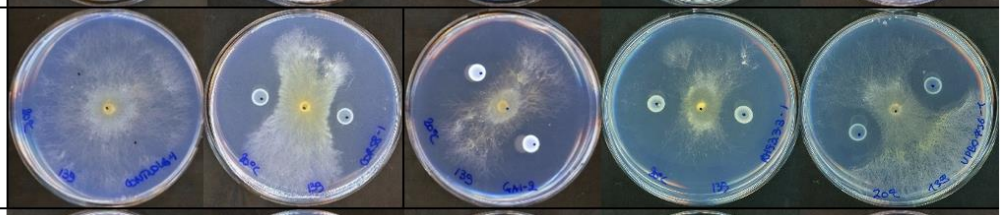


Figure A 2. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups three days incubation at 20 °C during *in vitro* antagonism test 1. The error bars indicate the standard deviation on the measurements. Within each isolate, there were no statistically significant differences found between different bacterial strains. Except for the dual culture of *R. solani* isolate 139 and bacterial strain UPB0736 which had three replicates, all dual cultures had four replicates.

Table A 1. A selection of representative pictures taken three days after incubation at 20 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 1.

Control/ Bacteria / Isolate	Control	COR58	GA1	RHF3.3-3	UPB0736
61 (AG-3)					
175 (AG-4 HGII)					
139 (AG-5-1)					
58 (AG-5-2)					
16 (AG-11)					

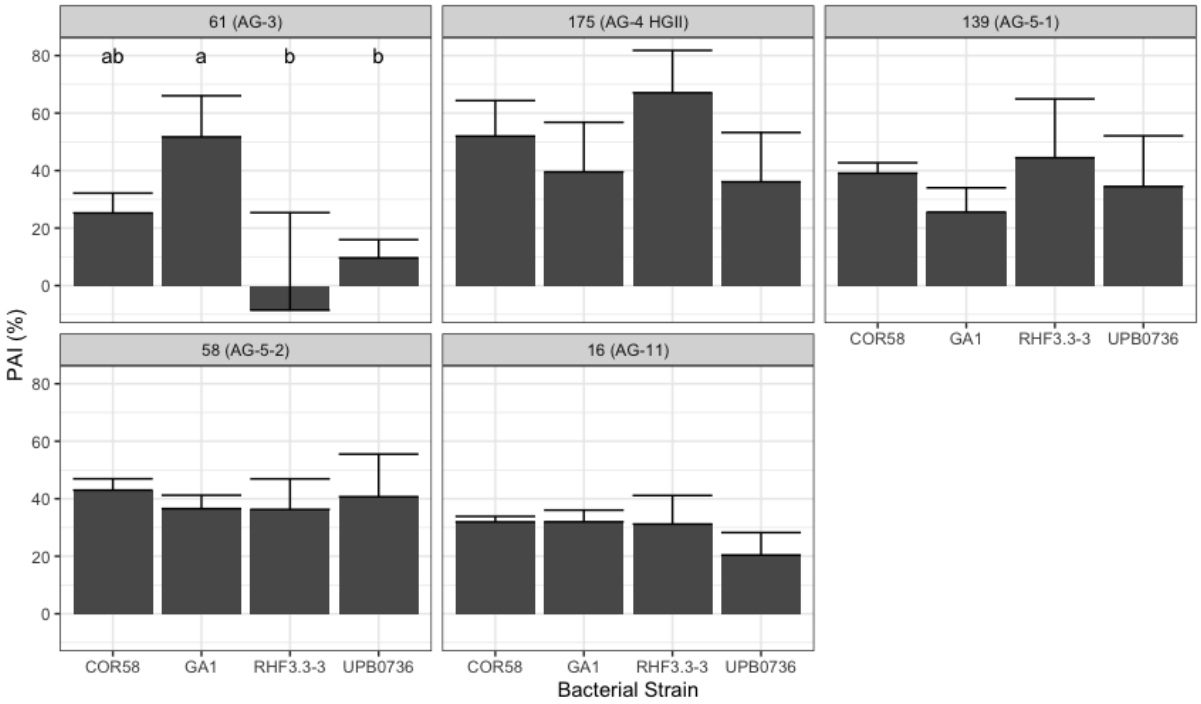


Figure A 3. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups three days after incubation at 28 °C during *in vitro* antagonism test 1. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. Except for the control plate of *R. solani* isolate 175 which had three replicates, all dual cultures and controls had four replicates.

Table A 2. A selection of representative pictures taken three days after incubation at 28 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 1.

Control/ Bacteria / Isolate	Control	COR58	GA1	RHF3.3-3	UPB0736
61 (AG-3)					
175 (AG-4 HGII)					
139 (AG-5-1)					
58 (AG-5-2)					
16 (AG-11)					

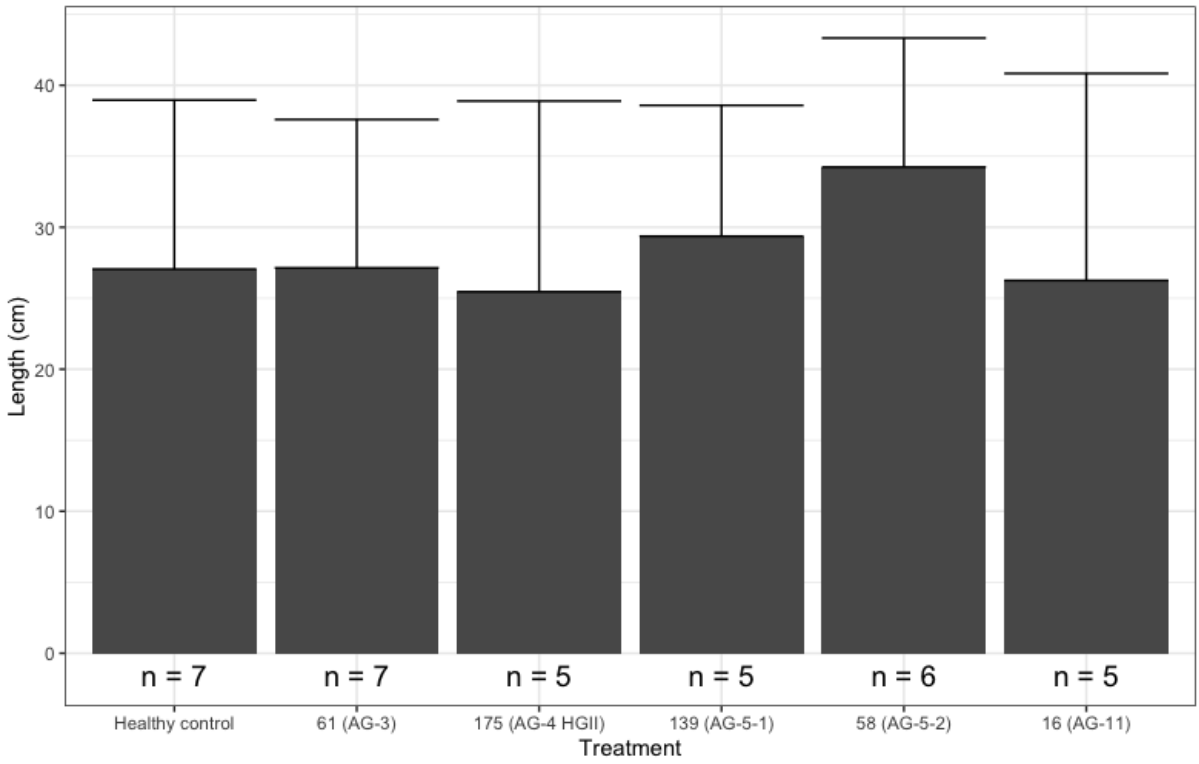


Figure A 4. The average length of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in the control batch in plant trial 3. The error bars indicate the standard deviations on the measurements. There were no statistically significant differences found between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

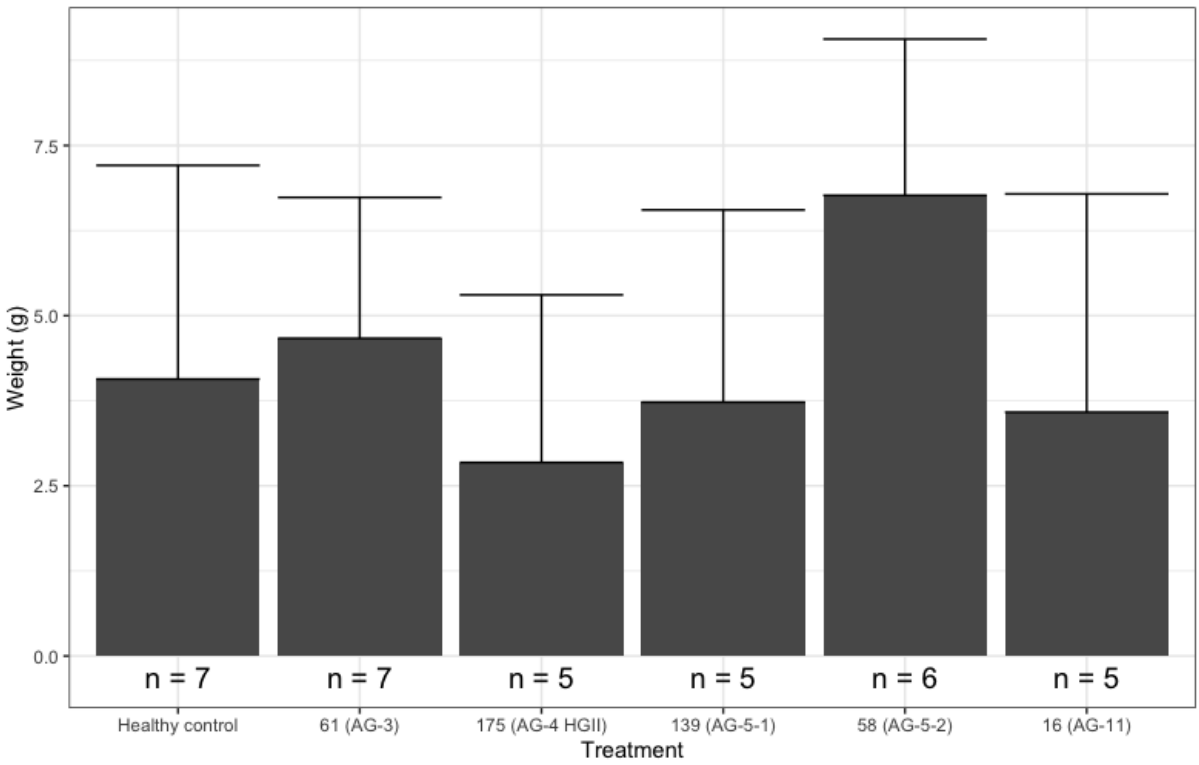


Figure A 5. The average weight of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in the control batch in plant trial 3. The error bars indicate the standard deviations on the measurements. There were no statistically significant differences found between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

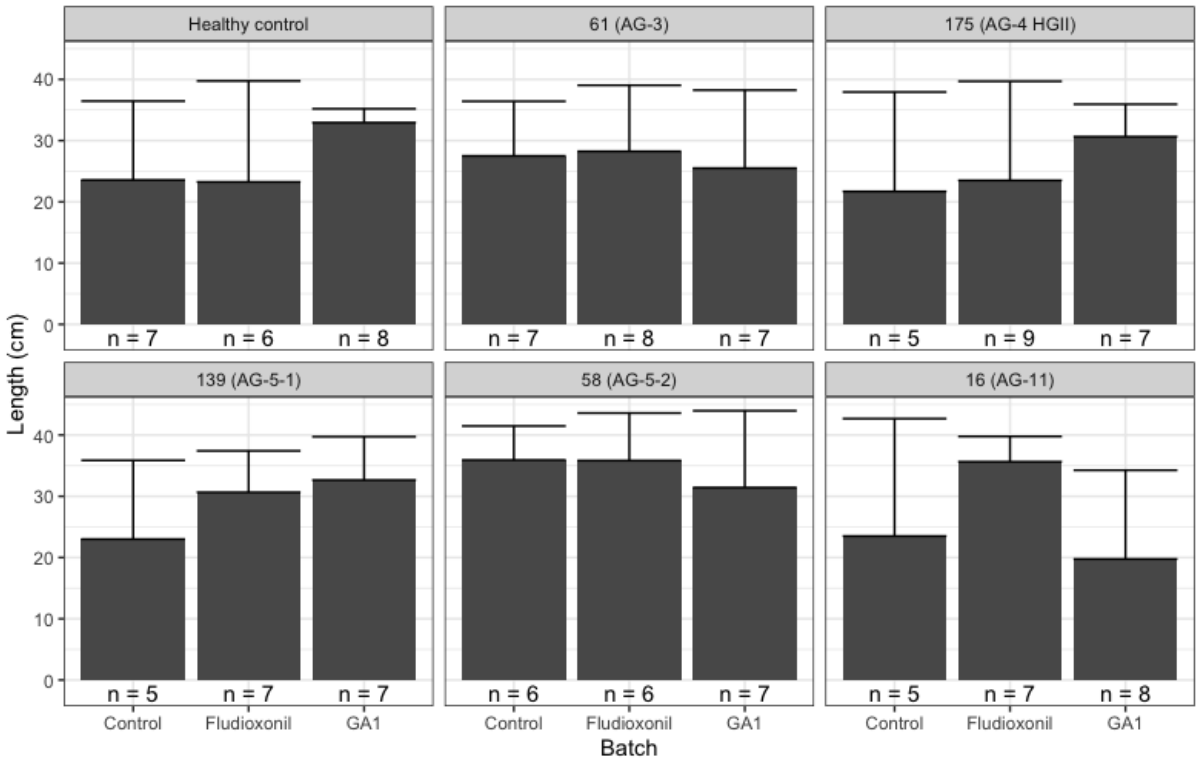


Figure A 6. The average length of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 3. The error bars indicate the standard deviations on the measurements. Within each isolate treatment, there were no statistically significant differences found between different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

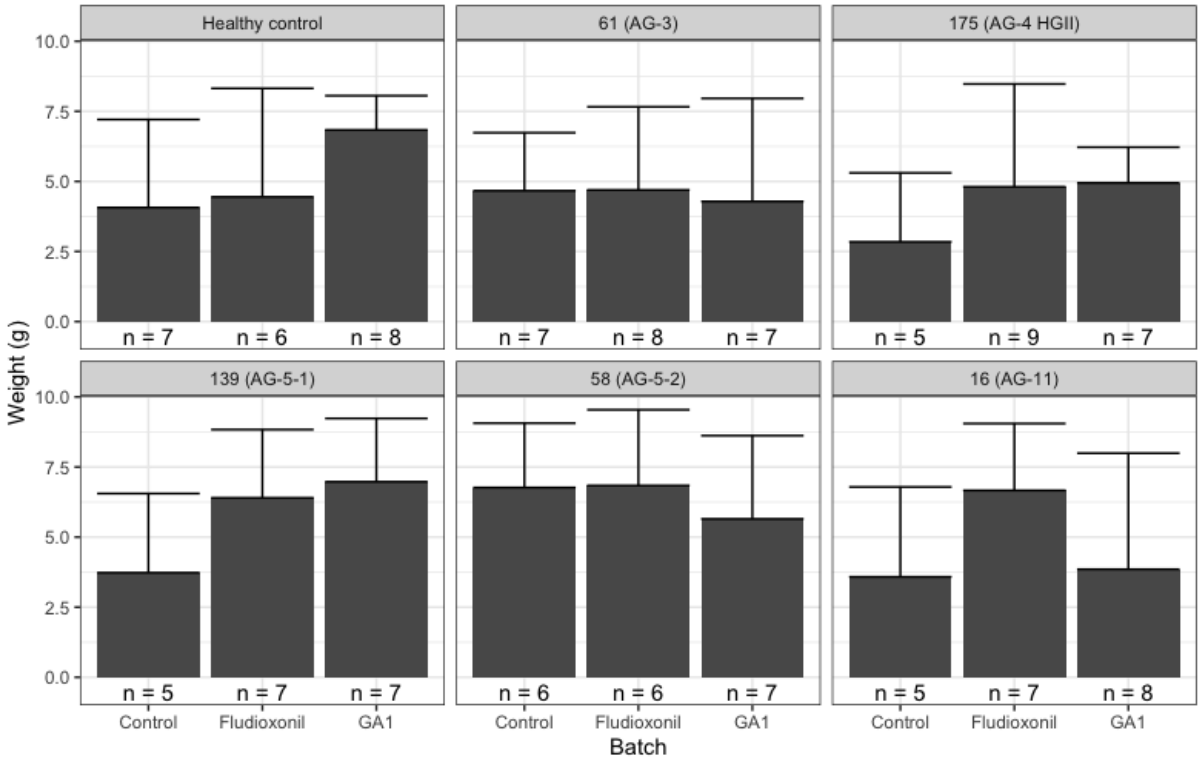


Figure A 7. The average weight of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in plant trial 3. The error bars indicate the standard deviations on the measurements. Within each isolate treatment, there were no statistically significant differences found between different batches. The number of plants (n) used in each treatment is given at the bottom of the graph.

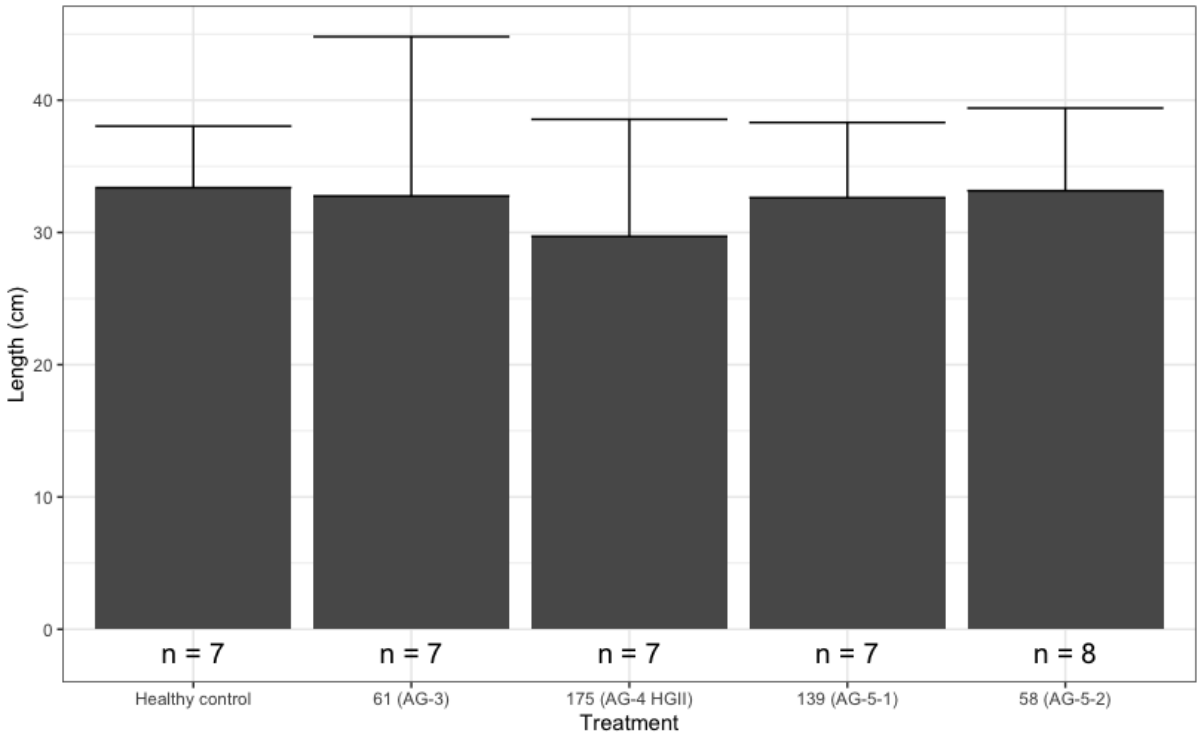


Figure A 8. The average length of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in the control batch in plant trial 4. The error bars indicate the standard deviations on the measurements. There were no statistically significant differences found between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

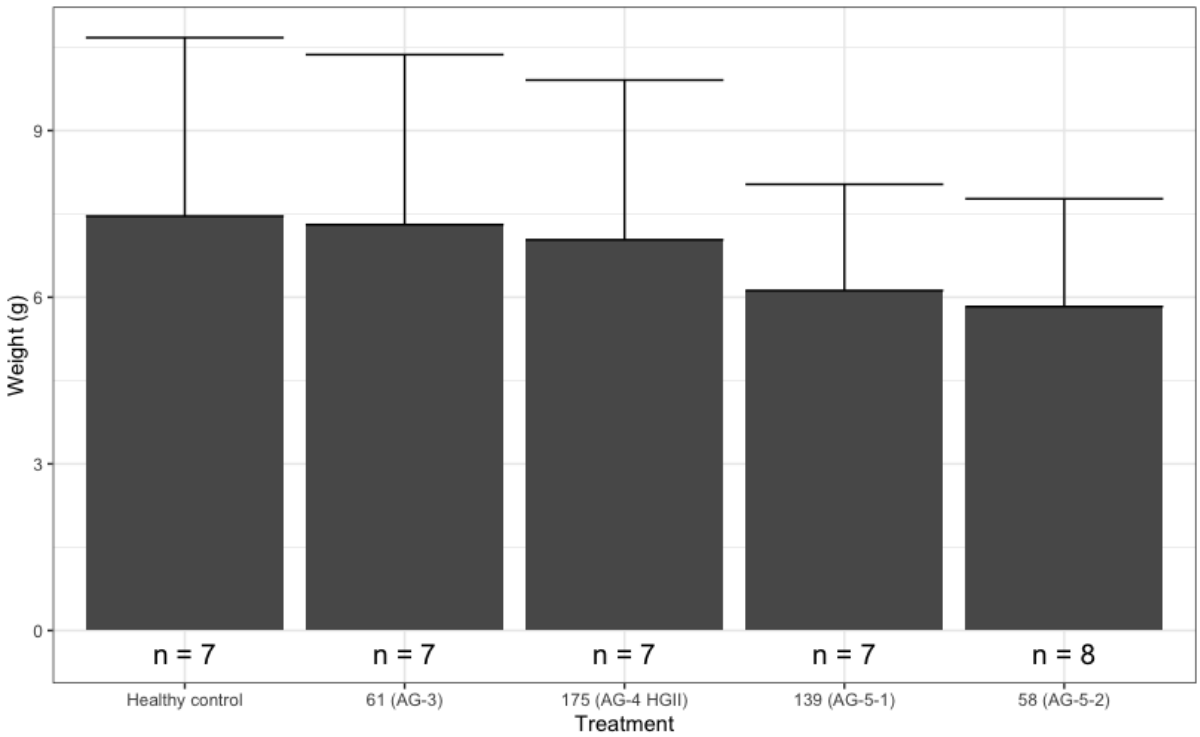


Figure A 9. The average weight of faba bean plants treated with *Rhizoctonia solani* isolates belonging to different anastomosis groups and a healthy control in the control batch in plant trial 4. The error bars indicate the standard deviations on the measurements. There were no statistically significant differences found between treatments. The number of plants (n) used in each treatment is given at the bottom of the graph.

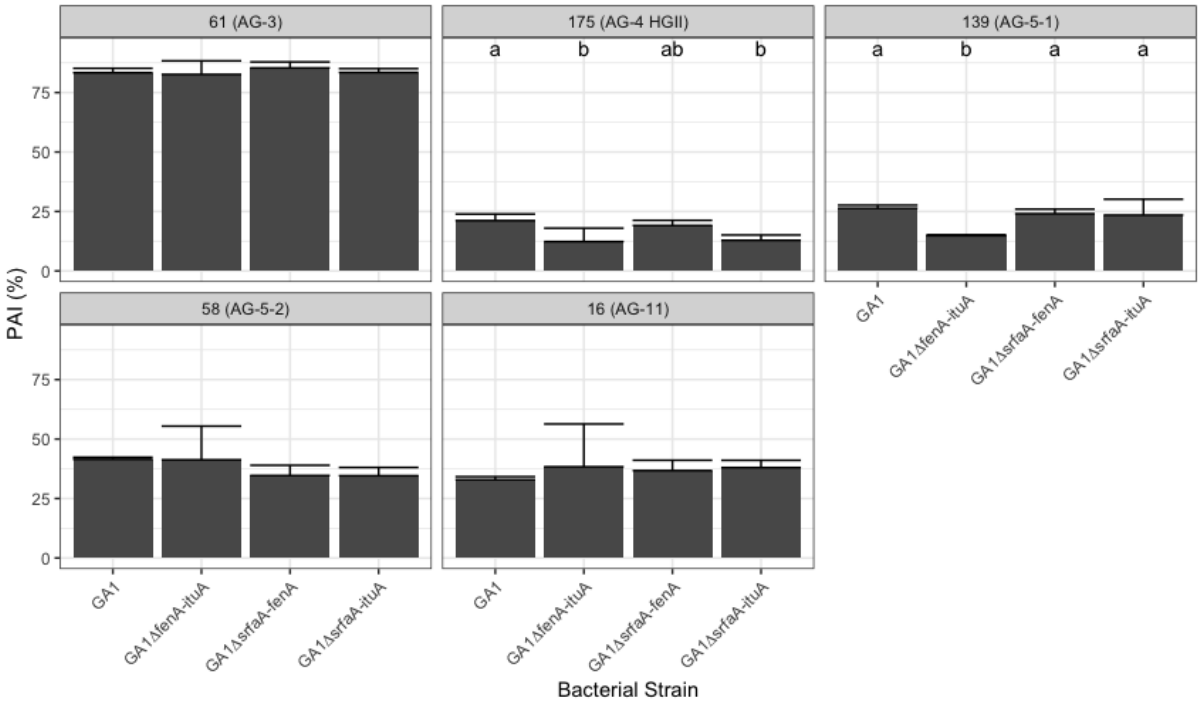
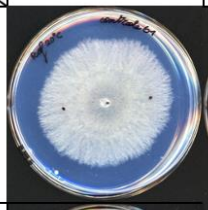
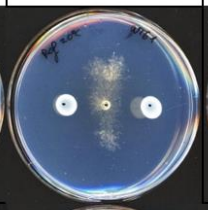
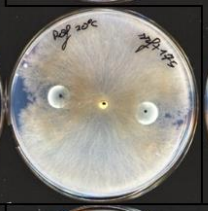
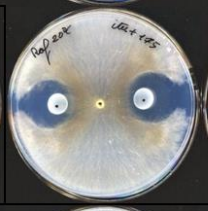
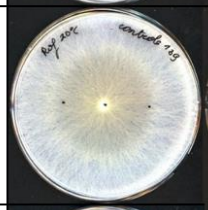
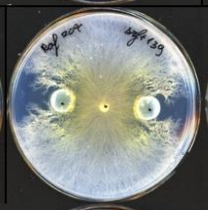
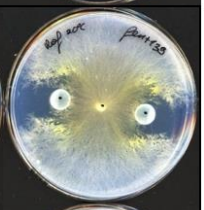
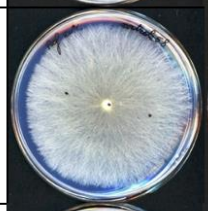
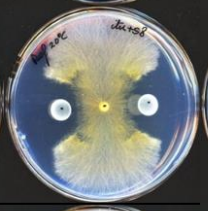
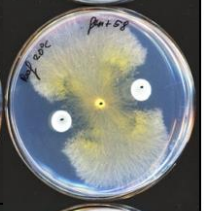
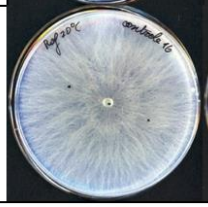
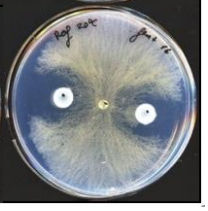


Figure A 10. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups three days after incubation at 20 °C during *in vitro* antagonism test 2. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. Except for the dual culture of *R. solani* isolate 58 and bacterial strain GA1ΔsrfaA-ituA which had three replicates, all dual cultures and controls had four replicates.

Table A 3. A selection of representative pictures taken three days after incubation at 20 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 2.

Control/ Bacteria / Isolate	Control	GA1	GA1ΔfenA-ituA	GA1ΔsrfaA-fenA	GA1ΔsrfaA-ituA
61					
175					
139					
58					
16					

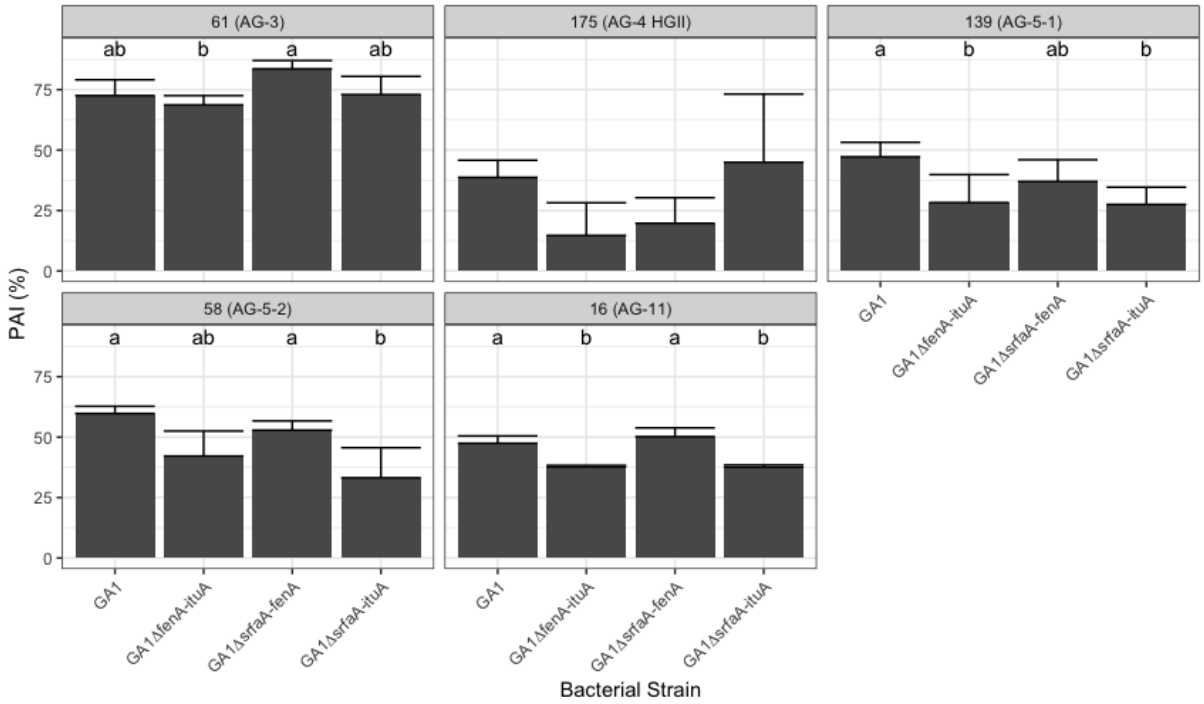
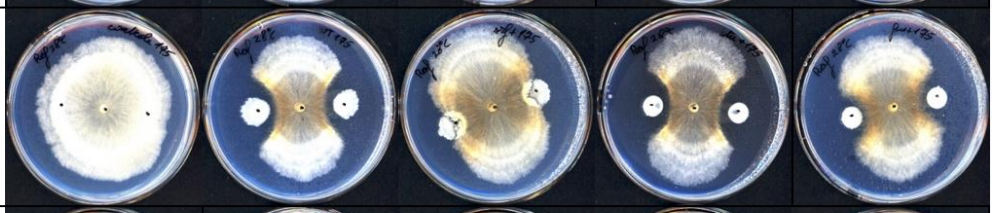


Figure A 11. Means of the Percentage Area Inhibition (PAI) caused by different bacterial strains of *Rhizoctonia solani* isolates belonging to different anastomosis groups three days after incubation at 28 °C during *in vitro* antagonism test 2. The error bars indicate the standard deviation on the measurements. Differing letters at the top of the graph indicate that within an isolate, there were statistically significant differences in PAI between bacterial strains. The data of isolates 175 and 16 were found to be heteroskedastic. Except for the dual culture of *R. solani* isolate 58 and bacterial strain GA1ΔfenA-ituA which had two replicates, all dual cultures and controls had four replicates.

Table A 4. A selection of representative pictures taken three days after incubation at 28 °C from the dual cultures of *R.solani* isolates belonging to different anastomosis groups and different bacterial strains during *in vitro* antagonism test 2.

Control/ Bacteria / Isolate	Control	GA1	GA1ΔfenA-ituA	GA1ΔsrfaA-fenA	GA1ΔsrfaA-ituA
61 (AG-3)					
175 (AG-4 HGII)					
139 (AG-5-1)					
58 (AG-5-2)					
16 (AG-11)