Aus dem Institut für Phytopathologie
der Christian-Albrecht-Universität zu Kiel

Field assessment of the influence of a seed treatment with the antagonistic bacterium *Serratia plymuthica* on the control of major rapeseed pathogens in *Brassica napus*

Dissertation

zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
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vorgelegt von

M.Sc. Daniel Marquardt
aus Oldenburg i.H.

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1. Introduction

1.1 The importance of oilseed rape

Cultivation of oilseed rape (*Brassica napus* L.) had its breakthrough in the 1970s due to the development of the "double-zero" rapeseed varieties (00-rapesseed), which have only small amounts of the bitter tasting erucic acid and are nearly free from glucosinolates. Previously these two toxic components prohibited the use of rapeseed for food purposes (oil and margarine) or as feed for pigs, cattle and chicken. Nowadays, with acreage of 5.4 million hectares (2007), rapeseed represents a major crop in the European Union and is the second largest oil crop worldwide (Gehringer et al. 2007).

Besides an increase in demand for biodiesel (rape methyl ester) and edible oil, oil seed rape is also attractive as a preceding crop. These were reasons of the development of the area under cultivation of constantly more than 1 million hectares in the last ten years in Germany. In 2010 almost 1.5 million hectares of rapeseed were grown (ufop 2011).

1.2 Oilseed rape diseases and control

The extension of rapeseed cultivation results in closer crop rotations and increased abundance of fungal diseases in growing areas. Economically relevant pathogens are *Leptosphaeria maculans* (Desmo.) Ces and de Not. (anamorph *Phoma lingam* Tode ex Fr.) (blackleg disease), *Verticillium longisporum* (ex. *V. dahliae var. longisporum* Stark, comb. nov. Karapana) (*Verticillium* wilt), *Sclerotinia sclerotiorum* (Lib.) de Bary (stem rot) and *Plasmodiophora brassicae* Woronin (club root). Whereas *P. lingam* and *S. sclerotiorum* can be controlled by application of fungicides, *V. longisporum* and *P. brassicae* are not affected by common chemicals. Furthermore, pathogens can develop resistance against applied agents and therefore biological control might offer an alternative to application of fungicides. Throughout the last decade, many naturally occurring soil microorganisms have been screened for their ability to control seed- and soil-borne diseases (Slininger et al.1998). Among several bacteria and fungi identified as biocontrol agents (BCAs), the most abundant soil and plant-associated bacteria are in the genera *Burkholderia, Bacillus, Pseudomonas, Serratia* and *Streptomyces* (Berg et al. 2002, Nair et al. 2002, Costa et al. 2006, Mark et al. 2006). BCAs operate as competitors for substrates and space and can have a direct antagonistic effect on the pathogens throughout the synthesis of antifungal or rather antibiotic metabolites (Chernin and Chet 2002). Most BCAs generate various active substances and so

their selective use minimizes the selection pressure on pathogens to develop resistance (Handelsman and Stabb 1996). The duration of a fungicidal effect of synthetic fungicides is limited and therefore applications must be repeated and timing is of great importance, whereas most micro organisms are able to establish at target locations and act continuously thus providing sustainable effects (Sivan and Chet 1992). Furthermore, plant growth promoting effects and the ability to induce resistance in plants increases the attractiveness of some BCAs (Harmann 2006).

Nevertheless, only few potential BCAs have been developed to reach market quality. Reason for that are technical problems (Whipps 2001) and the insufficient control by the antagonists under field conditions, which has been frequently described in the literature (e.g., Weller 1988; Gerhardson and Larsson 1991; Hornby et al. 1993; Tahoven et al. 1995). Another disadvantage of BCAs is the often limited range of pathogens that can be controlled by a single BCA (Cook 1993; Campell 1994). *Pseudomonas chlororaphis* isolate MA 342 represents an efficient counterexample as it provided consistent effects against several cereal diseases under field conditions (Johnsson et al. 1998). The active organism was developed by Lantmännen BioAgri AB (Uppsala, Sweden), is produced by e-nema GmbH (Schwentinental) and sold under the trademarks Cedomon® and Cerall®. Approximately 1.5 million hectares have been cultivated with seeds treated with MA 342 in Sweden since 1997 (Hökeberg 2006).

1.2.1 ***Phoma lingam***

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans*, is one of the most important diseases in oilseed rape and occurs in Central Europe, Australia, Canada and China (Fitt et al. 2006; Howlett 2004; West et al. 2001). Annual crop losses of more than 100 million Euros in European countries are possible, while losses at harvest are usually less than 10 %, although they can reach up to 30-50 % (Fitt et al.; West et al. 2001).

The life cycle of *P. lingam* is demonstrated in Fig. 1 *L. maculans* generates ascospores in pseudothecia located on infected residuals of previous crops, which are primarily released from September until November in Central Europe (Krüger and Wittern 1985; Pérès et al. 1999). These air-borne ascospores cause the primary infection by penetrating leaves of young seedlings after few hours of leaf moisture. First visual symptoms appear in the form of lesions containing distinctive asexual pycnidiospores (Fig. 2), which are spread out by the kinetic energy of impinging raindrops. In the following, the pathogen colonizes petiole, root

collar and stem through systemic growth, which may lead to stem and crown cankers (Fig. 3, 4) that cause yield loss (Hammond et al. 1985; Fitt et al. 2006).

Isolates of *P. lingam* can be differentiated into two *Leptosphaeria*-species, which are classified as *L. maculans* (A-Type, virulent, aggressive) and *L. biglobosa* (B-Type, non-virulent, non-aggressive) (Mendes-Peira et al. 2003; Koch et al. 1989; Johnsen and Lewis 1994; McGee and Petrie 1979). While *L. maculans* is associated with damaging stem canker, *L. biglobosa* causes mostly non-damaging upper stem lesions (West et al. 2001; Huang et al. 2005). In northern parts of Germany the aggressive type is more common (Koopmann and Hoppe 1998, Volke et al. 2000a, Volke et al. 2000b).

At the moment *P. lingam* is controlled by azole fungicides and cultivation of tolerant cultivars. However, resistance genes may become ineffective after intensive use, whereas application of fungicides may reduce disease severity but is also affected by weather conditions and has to be timed correctly (West et al. 2002; Li et al. 2003; Rouxel et al. 2003). Therefore the rhizobacteria-mediated biological control could be an environmentally friendly alternative to protect plants against *P. lingam*.

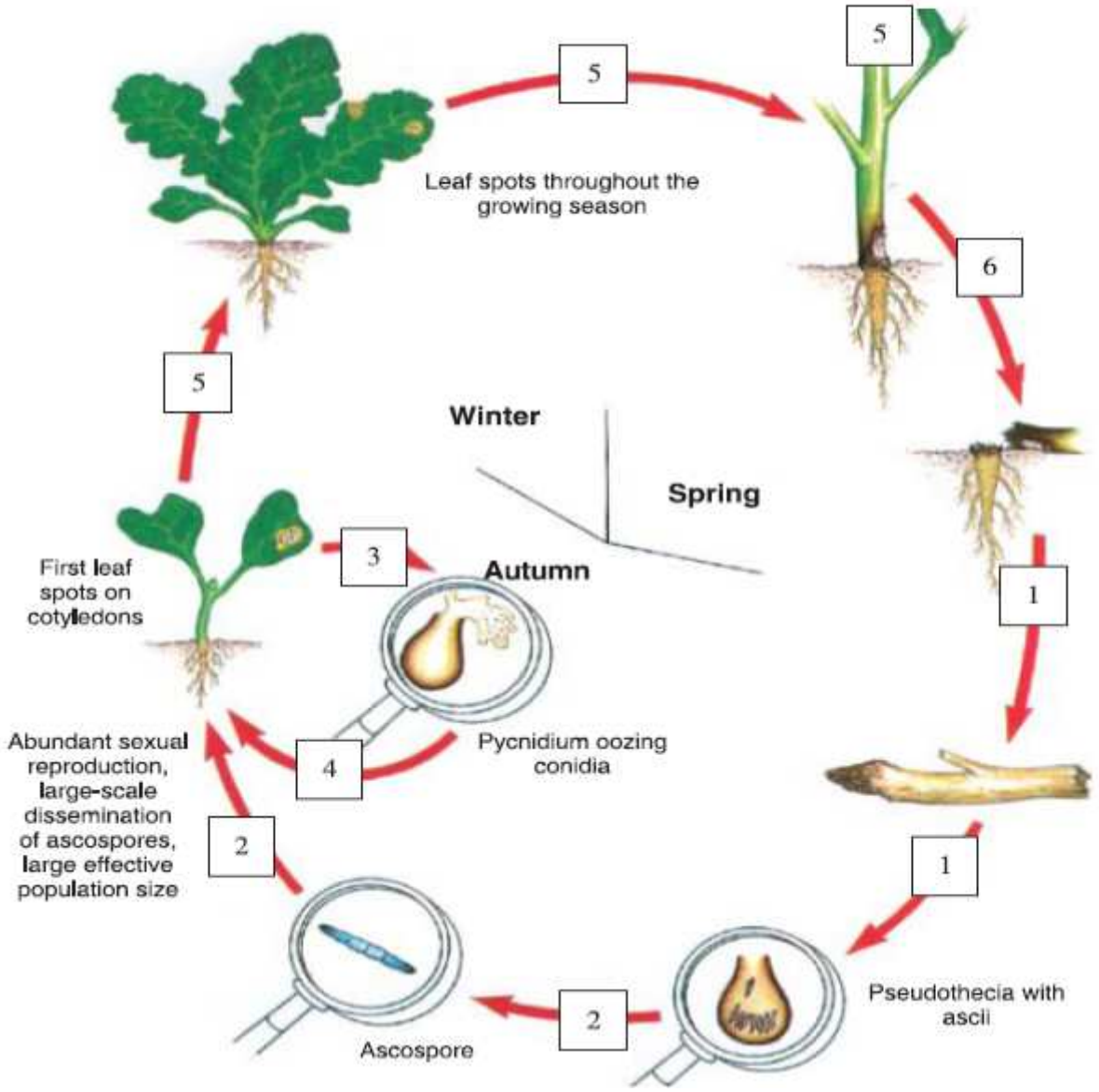


Fig. 1: Life cycle of *Phoma lingam* (Rouxel and Balesdent 2005)

Fig. 2: Lesions containing pycnidia on rapeseed leaves caused by *Phoma lingam*

Fig. 3: Crown cancer symptoms on rapeseed caused by *Phoma lingam*

Fig. 4: Stem infestation with pycnidia on rapeseed caused by *Phoma lingam*

1.2.2 *Verticillium longisporum*

The soil-borne fungus *Verticillium longisporum* is of worldwide distribution in temperate and sub-tropical regions and causes wilt on a broad range of crops (Steventon et al. 2002, Berg et al. 2001). Every year *V. longisporum* is responsible for yield losses of 10 to 50% in oilseed rape in Germany (Müller 2006). It survives more than 10 years in the form of thick walled and melanized microsclerotia in soil (Lucas 1998; Zeise and Seidel 1990; Collins et al. 2003). The life cycle of *V. longisporum* is demonstrated in Fig. 5. Germination of the microsclerotia is stimulated by root exudates of host plants (Wilhelm 1955; Schreiber and Green 1963). Winter oilseed rape can be infested two months past emergence of the seedlings, while typical half-sided yellowing of the older leaves usually occurs past flowering (Holtschulte 1992; Paul 1988). Hyphae of the fungus penetrate roots (Garber and Houston 1966) and the mycelium grows inter- and intracellular into the central cylinder. Damage is caused by blockage of the xylem through mycelium and/or conidia, as well as parasite-host-interaction induced, cell devastating metabolic products of the pathogen (Daebler et al. 1988; Niederleitner et al. 1991; Hoffmann et al. 1994; Lucas 1998). Initiated by senescence, typical microsclerotia are formed in the stem and mark before harvest (Fig. 6) (Günzelmann and Paul 1990; Krüger 1986; Zeise and Seidel 1999; Holtschulte 1992).

Control of the pathogen is limited to heat treatment and fumigation of the soil with broad spectrum biocides, such as methyl bromide (Berg et al. 2001), which is not practicable under large field conditions and prohibited in most countries. As chemical control is impossible, *V. longisporum* is becoming a major disease in some areas of Germany, France and other

European rapeseed-producing countries (Karapapa 1997). Therefore, the focus of breeders is on the development of resistant cultivars, since commercially available cultivars only possess a low level of resistance against the fungal disease (Rygulla et al. 2008). Preliminary glasshouse trials at the Institute of Phytopathology CAU Kiel (Germany) revealed that the BCA *S. plymuthica* strain HRO-C48 has potential to control *V. longisporum* in different oilseed rape cultivars.

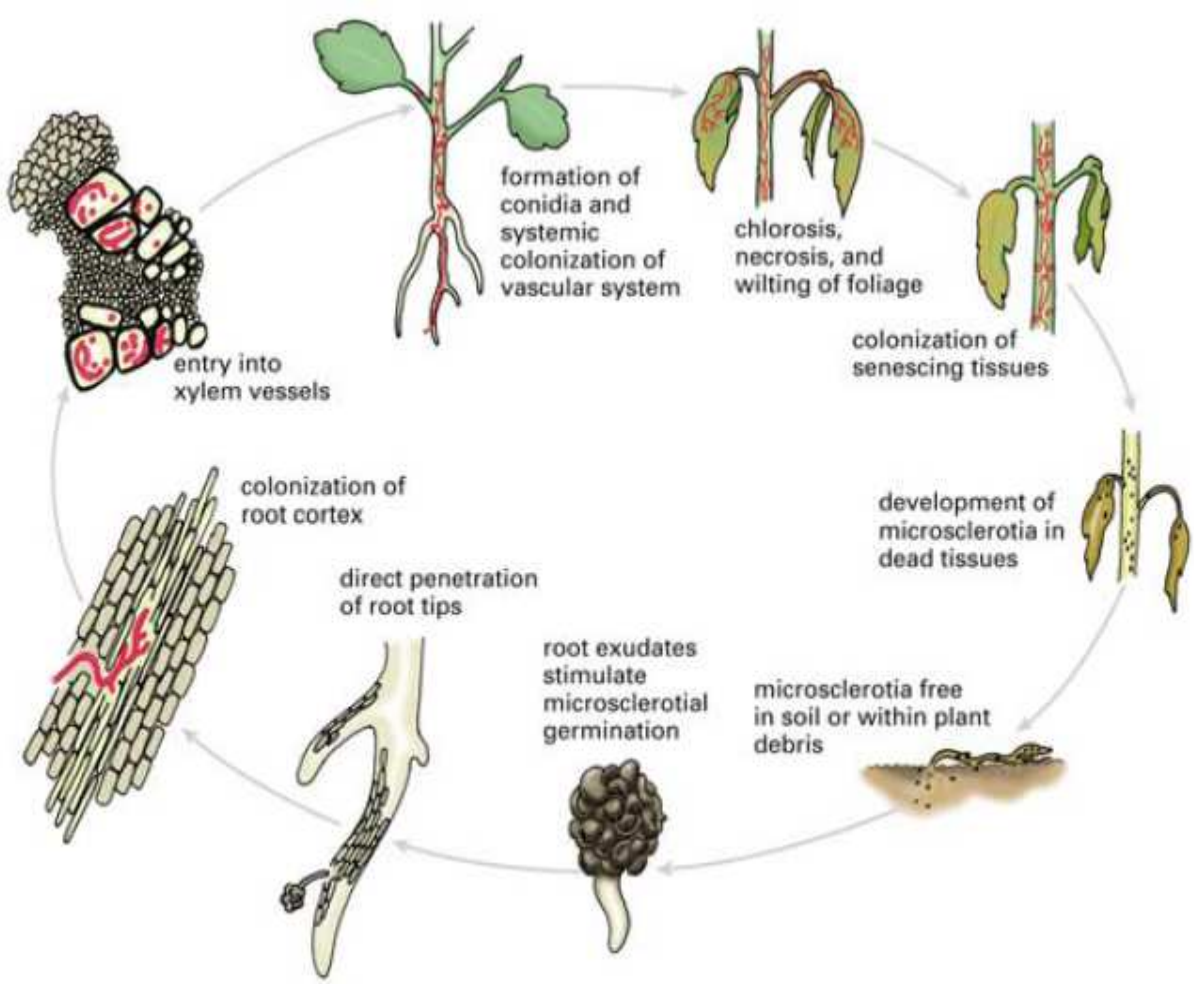


Fig. 5: Life cycle of *Verticillium dahliae* / *Verticillium longisporum* (Rowe and Powelson 2002).

Fig. 6: Infestation of rapeseed stem with microsclerotia of *Verticillium longisporum*

1.2.3 *Plasmodiophora brassicae*

Plasmodiophora brassicae is a soil-borne, obligate parasite causing clubroot disease, which is one of the economically most important and damaging diseases of *Brassica* crops. The fungal pathogen, recently regarded as a protist, infests more than 300 plant species including *Brassica rapa* (turnip rape, chinese cabbage), *B. oleracea* (cabbage, kohlrabi, kale), *B. napus* (rapeseed), *B. nigra* (black mustard) (Tjallingii 1965; Crute et al. 1983; Mattusch 1987) and a range of weeds. The amount of *P. brassicae* contaminated cultivation area is estimated to range up to 10 % globally (Crête 1981), while annual yield losses vary between 10 and 30 % depending on the affected crop (Ludwig-Müller 2000).

P. brassicae survives as resting spores in soil, which germinate into zoospores that penetrate root hairs of host plants. In the latter phase abnormal tissue proliferation in the root cortex takes place, forming galls (clubs) (Fig. 8). Inside these galls new resting spores are generated, which are liberated into the soil at the end of the life cycle (Ingram and Tommerup, 1972), which is shown in Fig. 7. The clubs formed inhibit nutrient and water transport, stunt the growth of the plant and increase susceptibility to wilting.

Cultural practices like multi-annual crop rotation and increasing the pH by lime conditioning can reduce the disease severity (Murakami et al. 2002). Effective soil fungicides are available (Tanaka et al. 1999), but agents do not have a registration in rapeseed in Germany, while application is expensive and not always successful. Therefore the development of resistant cultivars is now considered the most economical and efficient method for the control of

clubroot. Resistance has been identified in *B. rapa*, *B. oleracea* and *B. napus* (Crute et al. 1983; Crisp et al.1989). A few years ago Norddeutsche Pflanzenzucht (NPZ-Lembke) released the resistant cultivar Mendel that can tolerate moderate levels of resting spores.

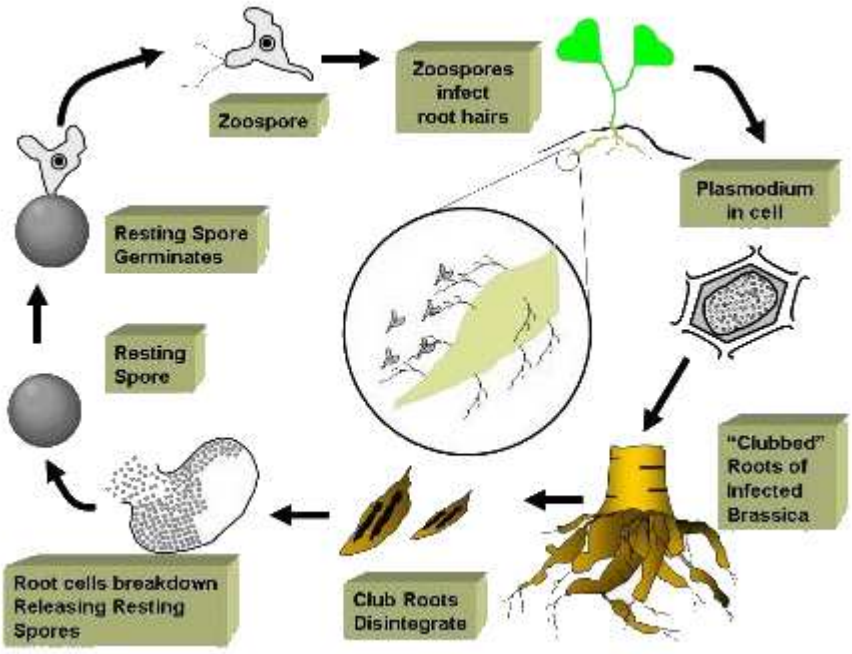


Fig. 7: Life cycle of *Plasmodiophora brassicae* (Manitoba Agriculture 2012)

Fig. 8: Clubs on roots of rapeseed caused by *Plasmodiophora brassicae*

1.2.4 ***Sclerotinia sclerotiorum***

The necrotrophic fungus *Sclerotinia sclerotiorum* is capable of infecting 408 plant species among 75 families including *Brassica napus* and *Phaseolus vulgaris* (Boland and Hall 1994). The ubiquitous pathogen manifests itself as stem rot in rapeseed, which is one of the most important diseases in Canada, where it causes yield losses of 5-100 % (Martens et al. 1994, Manitoba Agriculture 2002). In the United Kingdom losses of up to 50 % due to this pathogen have been reported (Pope et al. 1989), whereas incidence of up to 70 % in areas with short crop rotation were noticed in Germany, which might result in losses from 10-30 % (Dunker 2004).

The lifecycle of *S. sclerotiorum* is shown in Fig. 9. Vegetative sclerotia persist in the soil and germinate under moist conditions to form apothecia. The infection of rapeseed occurs either by the release of airborne ascospores from these apothecia (Abawi and Grogan 1975; Cook et al. 1975) or via mycelium targeting roots. Ascospores germinate on petals and infested petals- if lodged in leave axils -can infect the stem. Infection of stem and further spread of the disease solely occurs through growth of mycelium.

To date, complete resistance to this pathogen has not been identified, although partial resistance was recently reported in *B. napus* cv. Zhongyou 821 (Buchwaldt et al. 2003; Li et al. 1999). As there are no resistant cultivars available at the moment preventive application of fungicides at the time of flowering has become usual. However, research has shown that preventive application of fungicides is cost effective in one of three cases (Dunker 2006; Dunker and Tiedemann 2004; Wahmhoff 2000). Biocontrol might be an efficient and environmentally friendly alternative to control this pathogen (Köhl and Fokkema 1998). BCAs based on the fungal antagonists *Trichoderma harzianum* (Elad 2000) and *Coniothyrium minitans* (Li et al. 2005) reduced infestation of *S. sclerotiorum* in cucumber or alfalfa while strains of the bacteria *Pseudomonas fluorescens* and *P. putida* showed good control of *S. sclerotiorum* in sunflowers (Kamensky et al. 2002).

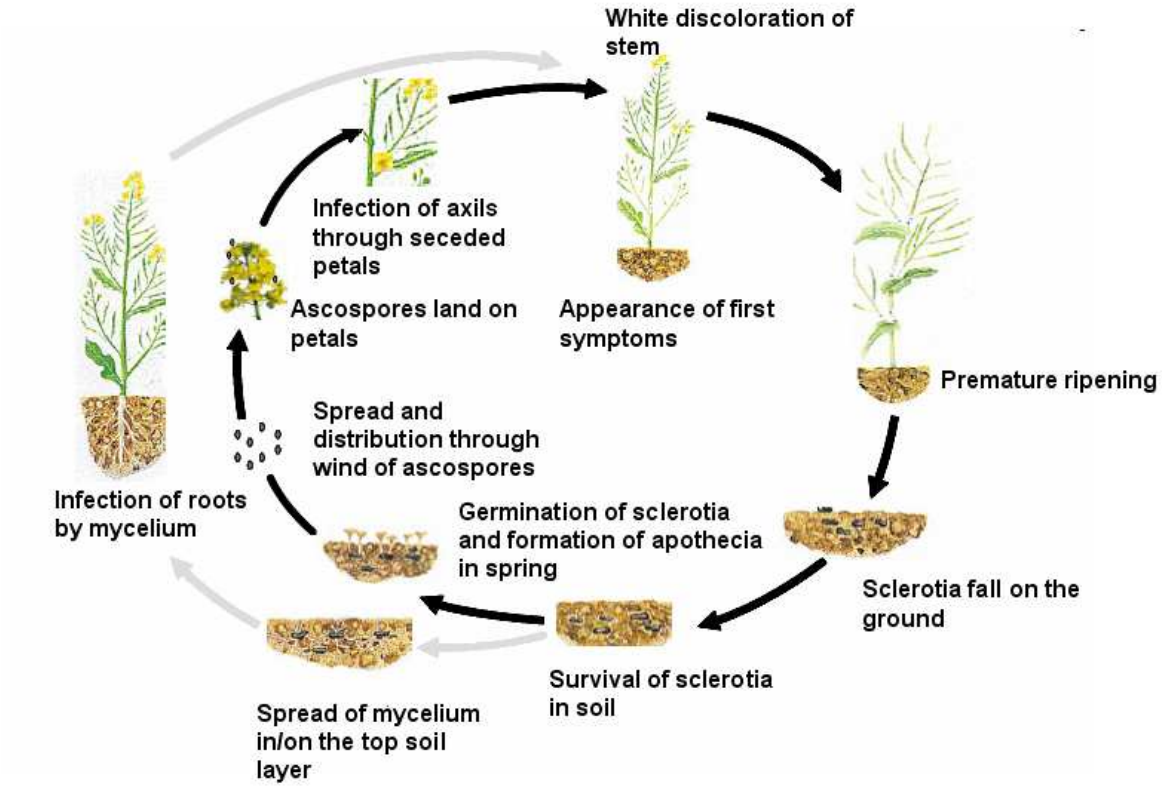


Fig. 9: Life cycle of *Sclerotinia sclerotiorum* (modified according to Paul 2003)

1.3 Use of antagonistic bacteria in seed treatment

High rhizosphere colonizing potential of crop plants is essential to obtain sustainable effects of soil borne antagonists (Weller 1988). Rhizosphere colonisation can be initiated by treating seeds with the antagonistic bacteria. As Gram-negative bacteria, like *S. plymuthica*, are unable to produce spores, their survival depends on a successful establishment of the bacterial cells in a protected environment, which is provided inside of a seed. To reach high bacterial concentrations inside the seeds within a short time, adapted application technology is necessary. According to Rhodes (1993) and (McIntyre and Press 1991) seed treatment should be fast and the procedure easily be integratable into the commercial seed treatment process. Technology needs to be adapted in order to protect the bacteria against various abiotic factors during storage and shipping (Jones and Burges 1998).

According to Müller and Berg (2008), bio-priming is one of the most effective methods to establish bacteria inside seeds. This technique is based on imbibtion by the seed, which leads to an uptake of bacterial suspension and enrichment of bacteria inside the testa. From bio-primed seeds bacteria have been re-isolated at densities of almost $\log_{10}$ 5 CFU seed^{-1} after 30 days of storage at 20°C. This procedure also improves germination (Gray, 1994). Through further investigation by Abuamsha et al. (2010b) the duration of the priming procedure could

be reduced from 12 to 2 hours, however, the following re-drying is still time consuming. Abuamsha et al. (2010b) did further research on this technique using *S. plymuthica* and *P. chlororaphis* and found out that addition of $MgSO_4$ supports the establishment of bacteria inside the seeds, while a temperature of 28°C offers the best conditions for the procedure of priming with *S. plymuthica*. Storage ability was prolonged by anaerobic conditions, whereas *P. chlororaphis* survived significantly longer than *S. plymuthica*, in general.

The so called "drum priming" (Rowse 1996), another application techniques for Gram-negative bacteria, finds use in seed treatment of leek, carrots and parsnip, but is not suitable for rapeseed. A further, widespread method for application of micro organisms is coating (pelleting), which can easily be integrated into the chemical seed treatment process (Burgues, 1998). Besides a reduced germination, coating can have a negative influence on the survival of bacteria (Farvel et al. 1998; Müller and Berg 2008). Another possibility to reach high bacterial concentrations within a short time was demonstrated by Schmidt et al. (2004), who put coated sugar beet seed into *Pseudomonas fluorescens* B5 (alternatively *P. corrugata* 2140) suspension for 15 minutes resulting in bacterial levels of $\log_{10}$ 3- $\log_{10}$ 9 CFU g^{-1} seeds.

In the process of chemical coating, talcum is added to absorb excessive liquid. This procedural step could be used to apply a bacterial-enriched binding agent. Vidhyasekaran et al. (1997) mixed several binding agents including talcum, kaolinite and lignite with carboxy-methylcellulose and added concentrated *P. fluorescens* suspension. This mixture was stored in sealed polyethylene bags for two days at 25°C. Bacteria from treated pigeon pea seeds (4g kg $seed^{-1}$) were able to colonize the roots at concentrations of $\log_{10}$ 5 CFU g^{-1} rhizosphere ninety days after sowing. Dandurand et al. (1994) also proved the survival of *Pseudomonas* spp. in different air-dried powdered minerals.

1.3.1 ***Serratia plymuthica*** HRO-C48

Serratia plymuthica strain HRO-C48 is a naturally occurring antagonistic soil bacterium which has originally been isolated from the rhizosphere of oilseed rape *Brassica napus* near Rostock (Germany) (Kalbe et al. 1996). An isolate-specific production of the chitinases CHIT60 and N-Acetylhexosaminidase (CHIT100) has been reported for this Gram-negative bacterium, which allows the entire degeneration of chitin into N-acetylglycosamin (Frankowski 2002). Furthermore the strain is able to produce the antibiotic agent pyrrolnitril, as well as several AHLs (*N*-Acyl-Homoserin-Lactone) like *N*-butanoyl-HSL, *N*-hexanoyl-HSL and *N*-3-oxo-hexanoyl-HSL (OHHL). These molecules are involved in „quorum-sensing" and have been reported to induce plant resistance (Liu et al. 2007).

HRO-C48 has a high antifungal activity against the fungal pathogens *Verticillium dahliae* subsp. *longisporum* (Kurze et al. 2001; Berg et al. 2000; Hammoudi 2007; Frankowski 2002), *Phytophtora cactorum* (Kurze et al. 2001), *Sclerotinia sclerotiorum, Rhizoctonia solani* and *Fusarium* spp. (Frankowski 2002). Another positive aspect is the production of indole-3-acetic acid (IAA) and its growth promoting effect has been reported in rapeseed and strawberries (Kurze et al 2001). Since 2005, *S. plymuthica* strain HRO-C48 is commercially applied as Rhizostar® by the e-nema GmbH (Schwentinental, Germany) to promote growth of strawberries.

Preliminary glasshouse trials at the Institute of Phytopathology CAU Kiel (Germany) revealed that *S. plymuthica* has potential to control *P. lingam* and *V. longisporum* in different oilseed rape cultivars under controlled conditions. In glasshouse trials with artificial *P. lingam* infection a mean reduction of the disease index (DI) of more than 70 % was observed for plants, which had been treated with *S. plymuthica* (Abuamsha et al. 2010a). Hammoudi (2008) showed significant reductions of moderate *P. lingam* leaf pycnidia as well as crown canker and stem disease scores through seed treatment with the antagonist in a field trial in northern Germany. Furthermore, HRO-C48 could reduce the area the under disease progress curve (AUDPC) of *V. longisporum* infested plants significantly by almost 60 % in average compared to the non-bacterial treated control, while approximately 70 % more healthy plants were obtained. Differences in control efficiency of the ten tested cultivars indicate a varietal dependence of the antagonist (Abuamsha et al. 2011).

1.4 Objectives

The main objective of this investigation was to test the performance of the bacterial antagonist *S. plymuthica* HRO-C48 against naturally occurring fungal rapeseed diseases in plot trials under field conditions and to find out whether the combined treatment with fungicides will result in an enhanced pathogen control. Furthermore, the colonization of the rhizosphere and bacterial population dynamics in different soil types and climates were recorded. Successful establishment is required for control effects (Abuamsha et al. 2010a, 2011; Hammoudi 2007).

Besides the field trials, the effect of HRO-C48 on *P. brassicae* and *S. sclerotiorum* under controlled conditions was of interest as well as the verification of the described control effect on *V. longisporum* in rape seed.

Another aspect of the investigation was the improvement of the seed treatment in order to further adapt the seed treatment process to practical needs for industrial use. Hence testing new methods as well as upgrading the existing procedure to enable integration into chemical rapeseed treatment was an objective of this study.

2. Material and Methods

2.1 Field trials

2.1.1 Trial sites

The fundamental idea while choosing the trial sites was to cover different soil types and climatic condition in northern parts of Germany with intensive rapeseed cultivation (Table 1). The locations shown in Fig. 10 were provided and cultivated by the German breeding companies Kleinwanzlebener Saatzucht AG (KWS), Norddeutsche Pflanzenzucht (NPZ-Lembke) and the agricultural institution Landwirtschaftskammer Schleswig-Holstein (LKSH) as well as the Christian Albrecht University (CAU) Kiel.

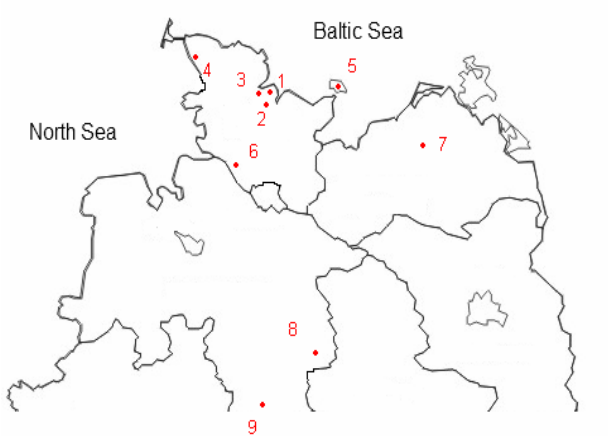


Fig. 10: Location of trial sites. 1=Birkenmoor, 2=Hohenschulen, 3=Hohenlieth, 4=Sönke-Nissen-Koog, 5=Sartjendorf, 6=Elskop, 7=Walkendorf, 8=Braunschweig, 9=Einbeck.

Table 1: Trial site information. AZ ("Ackerzahl", a parameter for soil quality reaching from 7 (very bad)-100 (very good)) as well as mean temperatures and mean annual precipitations of the trial episode 2008-2011. Information about soils and weather data were received from project partners: Kleinwanzlebener Saatzucht AG (KWS), Norddeutsche Pflanzenzucht (NPZ), Landwirtschaftskammer Schleswig-Holstein (LKSH) and Deutscher Wetterdienst (DWD).

No.	Site	Soil type	AZ	Temperature Ø (°C)	Annual precipitation (mm)
1	**Birkenmoor** (Schleswig-Holstein)	sandy loam	56	8,6	715
2	**Hohenschulen** (Schleswig-Holstein)	sandy loam – clay loam	55	8,1	744
3	**Hohenlieth** (Schleswig-Holstein)	sandy loam	56	8,7	838
4	**Sönke-Nissen-Koog** (Schleswig-Holstein)	silty loam	90	8,2	703
5	**Sartjendorf** (Schleswig-Holstein)	humic loam	73	8,9	636
6	**Elskop** (Schleswig-Holstein)	silty loam	65	7,7	843
7	**Walkendorf** (Mecklenburg-Vorpommern)	sandy loam – gleyey luvisol	51	8,6	588
8	**Braunschweig** (Niedersachsen)	loam	85	9,4	641
9	**Einbeck** (Niedersachsen)	loamy chernozem	90	8,9	739

2.1.2 Plant material

For the selection of the rapeseed cultivar the focus was on a high productivity and intensive cultivation in northern Germany. In field trials the hybrid Visby (Rapool) realised the highest yields in 2008-10 compared together rapeseed cultivars and is currently the most common cultivar (Rapool 2012). Visby is moderately resistant to the blackleg disease with a rating of 4 on a scale from 1 (low) to 9 (high resistant) (Bundessortenamt 2008).

2.1.3 Experimental design

Four different variants were chosen for the field trials (Table 2). Each variant had four randomly distributed replicates (plots). Each plot was divided into to identical sections, one for sampling and the other for yield assessment (except at Sönke-Nissen-Koog and Barlt).

Table 2: Variants of field trials

Variant	Term	Treatment
1	**Control**	**Seeds coated with Elado Premium and TMTD (flowering treatment)**
2	**Fungicide**	**Seeds coated with Elado Premium and TMTD Azole fungicide application in autumn, spring (+flowering treatment)**
3	**HRO-C48**	**Seeds coated with Elado Premium and TMTD + bio-primed with *S. plymuthica* HRO-C48 (flowering treatment)**
4	**Fungicide + HRO-C48**	**Seeds coated with Elado Premium and TMTD + bio-primed with *S. plymuthica* HRO-C48 Azole fungicide application in autumn, spring (+ flowering treatment)**

Cultivation practises such as fertilization, herbicides and insecticides (Table 3-5) were according to official recommended practise in the area concerned.

Table 3: Cultivation practises of trial sites 2008/09

	Birkenmoor	Hohenschulen	Hohenlieth	Sönke-Nissen-Koog	Braunschweig	Sartjendorf	Walkendorf
Plotsize	25 m^2	18 m^2	12,75 m^2	24 m^2	33 m^2	18 m^2	21 m^2
Seeding date	30.08.2008	31.08.2008	04.09.2008	20.08.2008	28.08.2008	29.08.2008	28.08.2008
Seeding rate	40 s/m^2	40 s/m^2	70 s/m^2	45 s/m^2	60 s/m^2	60 s/m^2	60 s/m^2
Previous crop	winter barley	winter barley	winter barley	winter barley	winter wheat	winter wheat	winter barley
Croprotation percentage rapeseed	25 %	33 %	20 %	25 %	16,6 %	25 %	21 %
Cultivation	plough; circular harrow	plough; disc harrow	plough; circular harrow	plough; circular harrow	no till	plough; circular harrow	plough; circular harrow
Fertilization	20 dt/ha Granukal 40 kgN/ha as KAS 95 kgN/ha as 15/9/20	210 kg N/ha as KAS 180 kg/ha Kieserit 0,5 l/ha Boric 140 8 kg/ha Epsom salts 1 l/ha Manganese-chloride	8kg/ha Micro Top 25 l/ha AHL 400 kg/ha ASS 10 kg/ha Microtop	no data available	200 kg/ha Triplephosphat 350 kg/ha 40er Kali 40 kgN/ha as KAS	no data available	no data available
Herbicides	30.08.08 2,0 l/ha Roundup Ultramax 01.09.08 2,5 l/ha Nimbus CS 29.09.08 0,7 l/ha Agill S	01.09.08 2,5 l/ha Nimbus CS 31.10.08 1,0 l/ha Focus Ultra	06.09.2008 2,0 l/ha Colzor Trio 10.10.08 0,75 l/ha Agil-S	29.08.08 0,3 l/ha Nimbus 25.09.08 0,25 l/ha Focus Ultra	07.09.08 2,0 l/ha Butisan Top + 0,5 l/ha Fusilade Top 25.09.08 0,4 l/ha Agil S	08.09.08 2,0 l/ha Butisan Top 13.10.08 2,0 l/ha Focus Ultra + 1 l/ha Solubor	28.08.08 2,5 l/ha Nimbus SC + 2,0 l/ha Round up max 24.03.09 0,35 l/ha Effigo + 1,5 l/ha Fusilade Max
Insecticides	29.09.08 75 ml/ha Karate Zeon 05.04.09 0,2 l/ha Trebon 30 EC 14.04.09 0,3 l/ha Biscaya 02.05.09 125 ml/ha Talstar 8 SC	06.04.09 0,2 l/ha Trebon 14.04.09 0,2 l/ha Trebon 08.05.09 75 ml/ha Karate Zeon	10.10.08 50 ml/ha Karate Zeon 04.04.09 0,3 l/ha Bulldock 09.04.09 0,125 l/ha Talstar 8 SC 15.04.09 125 l/ha Talstar 8 SC + 0,3 l/ha Biscaya	16.09.08 5 kg/ha snail granules 25.09.08 0,1 l/ha Talstar 03.05.09 0,3 l/ha Biscaya	03.04.09 100 ml/ha Fastac 18.04.09 0,3 l/ha Biscaya	08.09.08 0,3 l/ha Decis 13.10.08 0,1 l/ha Fastac	10.09.08 0,1 l/ha Fastac 20.09.08 75 ml/ha Karate Zeon 20.04.09 1,5 l/ha Reldan
Fungicides	07.10.08 0,75 l/ha	31.10.08 0,5 l/ha	08.10.08 0,5 l/ha	25.09.08 0,5 l/ha	17.10.08 1,0 l/ha	30.10.08 0,8 l/ha	10.10.08 0,5 l/ha

	Folicur 02.05.09 0,5 l/ha Cantus	**Caramba 06.04.09 0,6 l/ha Folicur 08.05.09 0,5 kg/ha Cantus Gold**	**Folicur 04.04.09 1,0 l/ha Folicur 06.05.09 0,8 l/ha Proline**	**Folicur 03.04.09 1,0 l/ha Folicur 03.05.09 0,5 l/ha Cantus Gold**	**Caramba 15.04.09 0,5 l/ha Caramba + 0,3 l/ha Moddus 27.04.05 0,5 l/ha Cantus Gold**	**Caramba 04.04.09 1,0 l/ha Caramba 09.05.10 0,5 kg/ha Cantus Gold**	**Caramba 07.04.09 0,75 l/ha Caramba 21.04.09 0,7 l/ha Caramba 11.05.09 0,5 kg/ha Cantus Gold**

Table 4: Cultivation practises of trial sites 2009/10

	Birkenmoor	**Hohenschulen**	**Hohenlieth**	**Sönke-Nissen-Koog**	**Braunschweig**	**Sartjendorf**	**Walkendorf**
Plotsize	**25 m^2**	**18 m^2**	**12,8 m^2**	**24 m^2**	**33 m^2**	**36 m^2**	**33 m^2**
Seeding date	**23.08.2009**	**21.08.2009**	**28.08.2009**	**20.08.2008**	**27.08.2009**	**26.08.2009**	**28.08.2009**
Seeding rate	**40 s/m^2**	**45 s/m^2**	**70 s/m^2**	**45 s/m^2**	**60 s/m^2**	**60 s/m^2**	**60 s/m^2**
Previous crop	**winter barley**	**winter barley**	**winter barley**	**winter barley**	**winter wheat**	**winter wheat**	**winter barley**
Croprotation percentage rapeseed	**25 %**	**33 %**	**20 %**	**25 %**	**16,6 %**	**16,6 %**	**32 %**
Cultivation	**plough; circular harrows**	**plough; disc harrow**	**plough; circular harrows**	**plough; circular harrow**	**no till**	**plough; circular harrows**	**plough; circular harrows**
Fertilization	**40 kg/ha DAP 90 kgN/ha as SSA+ KAS 70 kgN/ha as 15/9/20 20 kgN/ha as urea**	**180 kg N/ha 120 kg K_20/ha 80 kg P_2O_2/ha 40 kg S/ha 50 kg MgO**	**80-100 kg/ha P_2O_5 240 kg/ha K_2O 111 kg/ha KAS 346 kg/ha ASS 260 kg/ha KAS 10 kg/ha Microtop**	**100 kg/ha NPK 90 kg N/ha urea**	**180 kg/ha Triplephosphat 350 kg/ha 40er Kali 40 kgN/ha as KAS**	**250kg N/ha 80kg P/ha 80kg K/ha 50kg S/ha 3l Bor/ha 51 kgN/ha urea**	**2,4 dt/ha chicken dung 102 kgN/ha Optimag 86 kgN/ha KAS**
Herbicides	**25.08.09 2,7 l/ha Nimbus CS 30.09.09 0,5 l/ha Agil S**	**22.08.09 2,2 l/ha Nimbus CS**	**24.08.09 2,0 l/ha Round up turbo 08.09.09 2,0l/ha Butisan Top 13.10.09 0,75 l/ha Agil S**	**21.08.09 4,0l/ha Colzor Trio**	**6.09.09 2,0 l/ha Butisan Top + 0,5 l/ha Fusilade Max 25.09.09 0,4 l/ha Agil S**	**07.09.2009 2,0 l/ha Butisan Top**	**29.08.09 2,5 l/ha Nimbus SC + 1,5 l/ha Round up ultra max 20.09.09 0,9 l/ha Fusilade max**
Insecticides	**25.08.09 4 kg/ha Patrol Meta Pads**	**13.04.10 150 g/ha Trafo WG**	**21.09.09 75 ml/ha Karate Zeon**	**01.09.09 4 kg/ha Patrol Meta Pads**	**2.4.10 0,3l/ha Decis**	**19.05.10 0,1l/ha Fastac**	**20.09.09 75ml/ha Karate Zeon**

	25.09.09 4 kg/ha Patrol Meta Pads 30.09.09 50 ml/ha Karate Zeon 16.04.10 1,5 l/ha Reldan 22 26.05.10 75 ml/ha Karate Zeon		**13.10.09 0,3 l/ha Bulldock 12.04.10 125 ml/ha Talstar 8 SC 29.04.10 125 ml/ha Talstar 8 27.05.10 0,2 l/ha Mavrik**	**15.04.10 125 ml/ha Talstar 8 SC**	**16.04.10 0,1l/ha Fastac**		**10.04.10 0,1 l/ha Fatac**
Fungicides	**30.09.09 0,75 l/ha Folicur 07.04.10 0,75 l/ha Folicur 17.5.10 0,7 l/ha Proline**	**23.09.09 0,7 l/ha Carax 13.04.10 0,7 l/ha Carax 20.05.10 0,5 kg/ha Cantus Gold**	**21.09.09 0,5 l/ha Carax 12.04.10 1,0 l/ha Folicur 17.5.10 0,8 l/ha Proline**	**28.09.09 0,5 l/ha Folicur 08.04.10 1,0 l/ha Folicur 20.05.10 0,5 kg/ha Cantus Gold**	**28.10.09 0,8 l/ha Caramba 20.04.10 1,0 l/ha Caramba**	**30.10.09 0,8 l/ha Caramba 07.04.10 1,0 l/ha Caramba 20.05.10 0,5 kg/ha Cantus Gold**	**10.10.09 0,7 l/ha Caramba 10.04.10 0,7 l/ha Carax 15.5.10 0,7 l/ha Proline**

Table 5: Cultivation practises of trial sites 2010/11

	Birkenmoor	**Hohenschulen**	**Hohenlieth**	**Barlt**	**Braunschweig**	**Sartjendorf**	**Einbeck**
Plotsize	**25 m^2**	**18 m^2**	**12,8 m^2**	**11,25 m^2**	**33 m^2**	**24 m^2**	**21,6 m^2**
Seeding date	**20.08.2010**	**06.09.2010**	**08.09.2010**	**04.09.2010**	**06.09.2010**	**02.-04.09.2010**	**27.08.2010**
Seeding rate	**40 s/m^2**	**45 s/m^2**	**55 s/m^2**	**55 s/m^2**	**55 s/m^2**	**55 s/m^2**	**55 s/m^2**
Previous crop	**winter barley**	**winter barley**	**summer barley**	**winter wheat**	**winter wheat**	**winter wheat**	**winter wheat**
Croprotation percentage rapeseed	**25 %**	**33 %**	**20 %**	**25 %**	**16,6 %**	**25 %**	**0 %**
Cultivation	**plough; circular harrows**	**plough; disc harrow**	**plough; circular harrows**	**plough; circular harrow**	**no till**	**plough; circular harrows**	**plough; circular harrows**
Fertilization	**24 kg N/ha as 8-24-24-4 52 kg N/ha as SSA 60 kg N/ha 15-9-20 100 kg N/ha as KAS**	**210 kg N/ha as KAS 200 kg/ha Kieserit 1,5 l/ha Manganese-chelat 8 kg/ha epsom salts**	**170 kg N/ha**	**222 kgN/ha 175 kg K_2O/ha 45 kg P_2O_5/ha**	**180 kg/ha Triplephosphat 280 kg/ha 40er Kali 40 kg N/ha as KAS**	**140 kg N/ha**	**160 kg N/ha**
Herbicides	**21.08.10 3,0 l/ha**	**07.09.10 2,5 l/ha**	**09.09.10 1,8 l/ha**	**05.09.10 2,5 l/ha**	**27.09.10 2,0 l/ha**	**04.09.10 1 l/ha**	**10.09.10 2,0 l/ha**

	Nimbus CS 09.10.10 2,0l/ha Focus Ultra + 2,0 l/ha Dash	**Nimbus CS 10.10.10 1,0 l/ha Panarex 20.04.11 1,0 l/ha Lontrel 19.07.11 4,0 l/ha Biograde**	**Nimbus CS**	**Butisan Kombi + 0,25 l/ha CS 36 06.04.11 2,5 l/ha Focus Ultra**	**Butisan Top + 0,5 l/ha Fusilade Max**	**Colzor Trio**	**Butisan Top + 0,3 l/ha Agil**
Insecticides	**31.08.10 7 kg/ha Metarex TDS 09.10.10 3 kg/ha Mollustop 10.10.10 0,3 l/ha Bulldock 11.04.11 0,2 l/ha Trebon 20.4.11 0,2 l/ha Mavrik 10.05.11 0,3 l/ha Biscaya**	**20.04.11 0,2 l/ha Trebon**	**01.10.10 0,3 l/ha Decis 02.04.11 0,2 l/ha Trebon 16.04.11 0,2 l/ha Trebon 20.04.11 0,2 kg/ha Mospilan 06.05.11 0,2 l/ha Mavrik**	**06.05.11 75 ml/ha Karate Zeon**	**14.04.11 0,3 l/ha Biscaya + 0,2 l/ha Mavrik**	**30.05.11 150 ml/ha Fastac**	**10.09.10 0,3 l/ha Decis 13.04.2011 150 g/ha Plenum**
Fungicides	**02.10.10 0,75 l/ha Folicur 04.04.11 0,75 l/ha Folicur 10.05.11 0,7 l/ha Proline**	**10.10.10 0,5 l/ha Folicur 20.04.11 1,0 l/ha Folicur**	**26.10.10 0,75 l/ha Folicur 09.04.11 1 l/ha Folicur 06.05.11 0,8 l/ha Proline**	**10.4.11 0,6 l/ha Tilmor 06.05.11 0,5 l/ha Proline**	**25.10.10 0,5 l/ha Caramba 07.04.10 1 l/ha Caramba 29.04.10 0,5 l/ha Cantus Gold**	**12.10.10 0,5 l/ha Caramba 19.4.11 1,0 l/ha Caramba 30.05.11 0,5 l/ha Cantus Gold**	**23.09.10 0,5 l/ha Caramba 07.04.10 1 l/ha Caramba 29.04.10 0,5 l/ha Cantus Gold**

2.1.4 Assessment of ***Phoma lingam***, ***Verticillium longisporum*** and ***Plasmodiophora brassicae*** disease symptoms

Sampling started in September/October and was carried out every 2-4 weeks (depending on climate and stage of development), except for winter season when climatic conditions prohibited sampling. A minimum of 10 plants per replicate was collected and screened for *P. lingam* symptoms on leaves, root collar and stem depending on the progress of plant development (BBCH-stages).

Lesions and pycnidia of *P. lingam* on leaves were counted from September/October (cotyledon stage) to end of May (loss of leaves) by using a binocular. From April onwards *P. lingam* root collar- and stem symptoms were assessed (Table 6, 7), while present pycnidia were counted again. All data according to *P. lingam* are given in area under disease progress curve values (AUDPC) and illustrated in box plots, while given percentages refer to differences in means. AUDPC was calculated using the following equation:

AUDPC= $\sum((y_i+y_{ti+1})/2) \times (t_{i+1}-t_i))$, where yi is the disease severity (frequency of infestation; number of pycnidia; disease score) for the observation number i, t_i is the corresponding number of days of disease assessment (Campell and Madden 1990).

Table 6: Scoring scheme for disease symptoms at root collars caused by *Phoma lingam* on oilseed rape (according to Krüger 1982).

Scores	Symptom description
0	**No infestation**
1	**Small spots on the root collar surface**
2	**Small spots on the surface including minor corking and/or browning**
3	**Intermediate value**
4	**Corking at root collar is clearly visible, stem-clasping, but not deep or deep at one side and/or severe browning**
5	**Intermediate value**
6	**Deep necking at root collar, severe abundance of pycnidia**
7	**Intermediate value**
8	**Corking at root collar deep and severe. No/few linking(s) to roots**

Table 7: Scoring scheme for disease symptoms at stems caused by *Phoma lingam* on oilseed rape (according to Krüger 1982).

Scores	Symptom description
0	**No infestation**
1	**Small spots on the surface**
2	**Larger spots on the surface**
3	**Intermediate value**
4	**Larger spots that goes deeper into stem**
5	**Intermediate value**
6	**Deep spots with pycnidia, stem dried up and softened**
7	**Intermediate value**
8	**Broad, deep infested areas. Plant has died due to infestation**

V.longisporum symptoms were assessed after harvest. A minimum of 25 stubbles per replicate were collected resulting in a total number of 100 per variant, site and year. Disease scores were assessed according to Krüger (1986) by using a binocular (Table 8). Results are given as disease index (DI) calculated using the following equation:

DI= [(n0x0) + (n1x1) + … + (n8x8) / (Nx8)] x 100, where n0-n6= number of plants belonging to classes 0-6, and N= the total number of plants.

Table 8: Scoring scheme for disease symptoms by *Verticillium longisporum* on oilseed rape (according to Krüger 1986).

Scores	Symptom description
0	**No infestation**
1	**Slight (1-5 cm) and one-sided spreading of microsclerotia**
2	**Still slight spreading of microsclerotia (3-10 cm)**
3	**Intermediate value**
4	**Clear symptoms. Microsclerotia colonized 10-30 cm of the stem**
5	**Intermediate value**
6	**More than half of the stem is cluttered with microsclerotia**
7	**Intermediate value**
8	**Plant is completely cluttered with microsclerotia**

To investigate the infection of roots with *P. brassicae* all collected plants were cut off and assessed according to Sacristan (1996) and Fähling (2001) (Table 9). Data are given as disease index.

In spring/summer 2009 the DLR (Dienstleistungszentrum Ländlicher Raum) Rheinland-Pfalz carried out a field trial in Gondershausen (near Koblenz) to investigate the effect of a seed treatment with *S. plymuthica* on the infection with *P. brassicae*. The soil was naturally contaminated with resting spores of the fungal disease. An even distribution of spores was testified by the planting of susceptible brown mustard. Belinda, a spring rapeseed cultivar, was chosen for this trial. Untreated seeds (control) were tested against seeds bio-primed with HRO-C48. There were three fully randomized replicates for the control and four replicates for the HRO-C48 variant. Planting was carried out on the 25th of May, while sampling took place four times till the end of August. A total of 80 plants per replicate was examined according to Sacristan (1996) and Fähling (2001). Data are presented as disease index.

Table 9: Scoring scheme for disease symptoms by *Plasmodiophora brassicae* on oilseed rape (modified according to Sacristan (1996) and Fähling (2001)).

Scores	Symptom description
0	**No infestation**
1	**Only small bulges (Ø < 3 mm), mostly on lateral roots**
2	**Only small bulges (Ø 4-8 mm), mostly on lateral roots**
3	**Small bulges (Ø 9-14 mm) on lateral roots and tap root; > 90 % healthy roots**
4	**Several medium-sized tumours at tap root (Ø 15-22 mm), no symptoms on the stem; > 70 % healthy roots**
5	**Medium-sized tumours at tap root (Ø 23-29 mm), no symptoms on the stem; 40-70 % healthy roots**
6	**Medium-sized – severe tumours at tap root (Ø 30-35 mm), no symptoms on the stem; 20-40 % healthy roots**
7	**Severe tumours at tap root (> 30 mm), slight wilting of the stem; 2-20 % healthy roots**
8	**dead plant**

2.1.5 Assessment of ***Serratia plymuthica*** HRO-C48 concentrations in the rhizosphere

For the assessment of the bacterial densities in the rhizosphere composite samples of roots with adhering soil of each bacterial treated replicate were made and suspended in water. Sample weight (1-10 g) and added water (20-50 ml) varied depending on the stage of development. After 2h on the shaker (180 rpm) a serial dilution was made and 10 µl of suspensions were plated on TSA containing 100 µg ml^{-1} rifampicin. Depending on the sample weight and water amount the lower detection limit was $\log_{10}$ 2 - $\log_{10}$ 3 CFU g^{-1} rhizosphere. Bacterial densities were calculated as means of eight samples per date and site.

2.2 Glasshouse trials

2.2.1 Experimental design of ***Verticillium longisporum*** trials

The hybrid varieties Visby and Trabant (Rapool) as well as two non-commercial KWS-cultivars (KWS 63, KWS 136) were used. Trabant was chosen because of its good performance in previous *V. longisporum* experiments (Abuamsha et al. 2011), while KWS 63 is known to be more susceptible to this pathogen than KWS 136.

For soil infestation microsclerotia of *Verticillium longisporum* (Isolate ELV25) were incubated in Erlenmeyer flasks containing 250 ml of Czapek-Dox medium (Difco Laboratories, USA) on a rotary shaker at 140 rpm for two weeks at 25°C until a level of $\log_{10}$ 7 conidia ml^{-1} was reached. After adding another 200 ml of Czapek-Dox medium the microsclerotia were mixed with 4 l of autoclaved vermiculite (Daemmstoffe Sproekhovel, Germany; granulation 1-2 mm) and incubated at room temperature for four weeks. Potting soil ED 73 (Einheitserdewerk, Uetersen, Germany) was mixed with vermiculite and the infested vermiculite ($\log_{10}$ 6 microsclerotia g^{-1}) at the ratio 8:3:1 (v/v/v) (modified according to Abuamsha et al. 2011). Pots for healthy controls were filled with potting soil mixed with vermiculite (4:1, v/v) (Müller and Berg 2008).

The experiments were carried out in a glasshouse (spring/summer) using artificial light (14 h photoperiod) in addition. Pots (9x9x9.5 cm), containing two plants, were placed on trays in a randomized design and watered and fertilized regularly. The experiment was conducted two times and contained 14 replicates of each treatment as follows: (1) healthy control (non-infested soil and no antagonists); (2) pathogen control (*V. longisporum* infested soil and no antagonists); (3) *S. plymuthica* (*V. longisporum* infested soil and HRO-C48 treated seeds).

The disease assessment of *V. longisporum* glass house trials according to Zeise (1992) started with occurrence of the first symptoms and was carried out twice a week till plateau phase was reached (Table 10). Afterwards bacterial densities in the rhizosphere were determined.

Table 10: Scoring scheme for disease symptoms at root collars induced by *Verticillium longisporum* on oilseed rape (according to Zeise 1992).

Scores	Symptom description
0	**No symptoms**
1	**Slight symptoms on oldest leaf (yellowing, black veins)**
2	**Oldest leave with strong symptoms (nearly dead)**
3	**Loss of the oldest leave**
4	**About 50% of leaves with strong symptoms**
5	**Loss of about 50% of leaves**
6	**Loss of over 50% of leaves**
7	**Only vegetation conus left**
8	**Dead plant**

2.2.2 Experimental design of *Plasmodiophora brassicae* trials

For testing the performance of HRO-C48 against *P. brassicae* the cultivars Visby and Mendel were chosen. Mendel, bred by Rapool, is the only cultivar that has a certified cultivar specific resistance against this fungal disease (Rapool 2011).

Resting spores of *P. brassicae* were extracted from clubs of infested plants collected from fields in northern Germany and their concentration was adjusted to $\log_{10} 5$ as well as $\log_{10} 7$ spores ml^{-1}, using tap water. In order to decrease the soil pH and promote the infestation a soil (ED 73) sand mixture was produced containing 5 % sand. Every seedling was inoculated with 3 ml of the particular resting spore suspension seven days after sowing by use of a pipette (modified according to Sacristán (1996) and Fähling (2001)). The experiments were carried out in a glasshouse using additional artificial light (14 h photoperiod). Pots (9 x 9 x 9.5 cm), containing four plants, were placed on trays in a randomized design and watered and fertilized regularly. The experiment was conducted three times and contained 60 replicates of each treatment as follows: (1) pathogen control (*P. brassicae* inoculation and no antagonists); (2) *S. plymuthica* (*P. brassicae* inoculation and HRO-C48 treated seeds).

The disease assessment of *P. brassicae* was done according to Sacristán (1996) and Fähling (2001) and the determination of bacterial densities in the rhizosphere took place seven weeks after starting the experiment (Table 9).

In addition, the DLR carried out a test series in cooperation with the Institute of Phytopathology using seeds of the variety Visby treated with *S. plymuthica* HRO-C48. For

this trial, seedlings were inoculated with *P. brassicae* concentration levels of 0, 10, $\log_{10}$ 2, $\log_{10}$ 3, $\log_{10}$ 4, $\log_{10}$ 5, $\log_{10}$ 6 and $\log_{10}$ 7 resting spores ml^{-1}. Pots with a volume of 800 ml were filled with approximately 450 g of potting soil. Eight plants per pot were sowed and inoculated with 2.5 ml of spore suspension each, after formation of the second cotyledon was completed. The disease assessment was also carried out according to Sacristán (1996) and Fähling (2001) (Table 9) eight weeks after sowing.

2.2.3 Experimental design of *Sclerotinia sclerotiorum* trials

Visby, Trabant and the line variety Lorenz were used. Trabant and Lorenz were chosen because of their good performance in previous experiments (Abuamsha et al. 2010a, 2011). According to the Bundessortenamt Visby is slightly more susceptible (5) to *S. sclerotiorum* than Lorenz and Trabant (6).

The plants were grown in pots (9x9x9.5 cm) in a greenhouse for 6-8 weeks under long day conditions. The mycelium of the fungal pathogen, originally from Mei et al. (2010), was incubated on PDA for 48-72 h before mycelium plugs (Ø 6 mm) were cut out by a cork borer. The biggest two leaves of every 6-8 week old plant were infested with two plugs each by pressing them upside down on the leave and fixing them by cling film (Fig. 11). Developing lesions on the leaves were measured after 12, 24, 36 and 48 h by using a calliper. Afterwards bacterial densities in the rhizosphere were assessed. Data on the development of lesions were AUDPC transformed

The experiment was conducted three times and contained 15 replicates of each treatment as follows: (1) pathogen control (*S. sclerotiorum* inoculation and no antagonist); (2) *S. plymuthica* (*S. sclerotiorum* inoculation and HRO-C48 treated seeds).

Fig. 11: Infection of a rapeseed leaf via *Sclerotinia sclerotiorum* mycelium plug

2.3 Seed treatment

2.3.1 Seed treatment for field and glasshouse trials

For an easy identification and re-isolation of the bacteria from seeds and root-samples a spontaneous rifampicin-resistant mutant of *S. plymuthica* HRO-C48 was used (Hammoudi 2007). Stock cultures had been stored at 80°C in Eppendorf-tubes filled with TSB (tryptic soy broth; bioMerieux Deutschland GmbH, Nürtingen) including 15 % glycerol. The bacteria were grown in liquid medium containing TSB 30g l^{-1} for 48 h at 180 rpm and 25°C until a level of $\log_{10}$ 10 - $\log_{10}$ 11 CFU ml^{-1} was reached. Afterwards, seeds were added to the liquid culture (1g $1ml^{-1}$) and left for 6 h on the shaker (180 rpm). Imbibition by the seeds leads to an uptake of the bacterial suspension and enrichment of $\log_{10}$ 6 - $\log_{10}$ 7 CFU $seed^{-1}$. Then the seeds were spread on trays covered with paper towels and dried for 12 h at 25 °C (Fig 12).

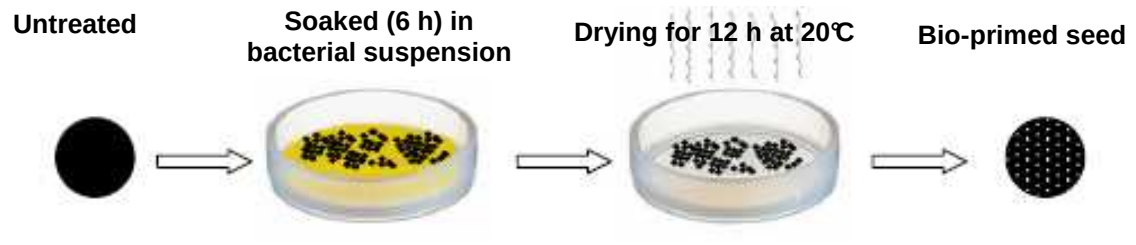


Fig. 12: Bio-priming procedure

To assess the bacterial density inside the seeds, three samples of 10 seeds were taken and grinded in 1 ml 0.85 % NaCl using a sterile mortar and pestle. Then a dilution series was made and suspensions were plated on TSA containing 100 µg ml^{-1} rifampicin. The agar–plates were incubated for 48 h at 25°C before colony forming units were counted and concentration could be calculated.

Seeds that were used for glass house trials were planted within 3 days after treatment, while bacteria-treated seeds and untreated seeds needed for field trials were chemically coated with Elado Premium (Clothianidin, beta-Cyfluthrin, Thiram, Dimetomorph) and TMTD by the NPZ (Hohenlieth, Germany) and were stored for a maximum of three weeks at 4-10°C before sowing.

2.3.2 Experimental design of seed treatment improvement

2.3.2.1 Spray application procedure

Bacterial suspension was applied to seeds by use of an airbrush gun. Two different concentrations of bacteria ($\geq\log_{10}$ 10 CFU ml^{-1} and $\geq\log_{10}$ 11 CFU ml^{-1}) and three different ratios of seeds and bacterial suspension (1:1; 1:2; 1:3 g seeds/ml bacterial suspension) were used. Before treatment samples of two grams of rape seeds were dried at 40°C until 5.8 %, 4.8 % and 2.8 % of moisture was obtained. After the application remaining suspension was dabbed off with paper towels and seeds were transferred to Petri dishes. For storage four different variants were chosen: V1) sealed for five days (Parafilm®) and stored cold (4°C); V2) opened (no lid) and cold; V3) sealed and stored warm (25°C); V4) opened and warm. Bacteria were re-isolated from seeds 2, 7 and 30 days after treatment (see 2.3.1).

2.3.2.1 Clay mineral procedure

The grinded clay minerals talcum (Carl Roth GmbH + CO. KG, Karlsruhe, Germany) and Vermiculite (e-nema GmbH, Schwentinental, Germany), as well as the diatomaceous earth Diafill (e-nema) were enriched with bacterial suspension ($\geq\log_{10}$ 11 CFU ml^{-1}) and afterwards applied within the chemical priming process. At first, 1 g of carboxyl-methyl-cellulose each was mixed with 99 g of the autoclaved clay minerals to be used as inert carrier. Then 10 ml of the bacterial suspension were added to the prepared carrier substances and mixed with the help of a grinder. Afterwards 14 Petri dishes per clay mineral were filled with 2.5 g of the substrate, sealed with Parafilm® and incubated at room temperature for 24 h. Next Petri dishes were opened again and dried for one, two, three, four and five hours. Then

one half of the sealed samples were stored at 25°C, the other half at 5°C. For re-isolation at 2, 7 and 30 days after treatment, 0.5 g of the samples were mixed with 1 ml 0.85 % NaCl in a 2 ml Eppendorf tube.

One kilogram of seeds (Visby) each was chemically coated with 10 ml Elado Premium (Clothianidin, beta-Cyfluthrin, Thiram) 10 ml DMM and 10 ml TMTD while 25 g of the bacterial enriched clay minerals (n2; stored <24 hours) were used to bind liquid. The procedure was carried out in a small seed treatment drum at Norddeutsche Pflanzenzucht (NPZ). Afterwards seeds were transferred to Petri dishes, sealed and stored at 25°C (5°C). Re-isolation of bacteria from seeds (see 2.3.1) was done two days after treatment.

2.4 Statistical analysis

Statistical analysis was computed with the software ***R*** (R Foundation for Statistical Computing, Vienna, Austria). All data recorded during field trials on infection with *P. lingam* and *V. longisporum* and data on *S. sclerotiorum* from glasshouse trials, were AUDPC transformed for the statistically analysis. Data on the effect against *P. brassicae* from glasshouse experiments and *V. longisporum* field trial data were transformed to disease scores, while *P. brassicae* field trial results are given as disease indexes. In cases when normal distribution was not given, data were assumed to be heteroscedastic. If additional random effects occurred, mixed models were applied, otherwise linear models were used. For field trial data an artificial factor (SY), including all combinations of trials-site and year was created, since trial sites changed in the last investigation period. Models were tested by analysis of variance (ANOVA). Depending on significant interactions of influencing variables, variants were compared, pooled and/or separately analysed by related multiple contrast tests at level $\alpha=0.05$. Regression between the intensity of leaf infestation and crown canker was verified by analysis of covariance (ANCOVA). Statistical significances regarding the improvement of seed treatment were calculated using the Tukey-HSD Test ($\alpha=0.05$) with XLSTAT (Microsoft) after analyses of variance using Sigma Plot (Systat Software Inc., San Jose, USA).

3. Results

3.1 Field trials

3.1.1 *Phoma lingam*

The ANOVA revealed significant interactions between trial site, year (SY) and variant (V) for leaf infestation and crown canker with p-values ranging between 0.0023 (F=1.9; df=60, 252) and $<\log_{10}$-16 (F=35.4; df=60, 252). On the other hand data of stem disease score and frequency of stem infestation showed no significant interaction between SY and V. Due to homogeneity and comparability of the results, multiple contrast tests of the different variants has just been conducted within the different sites and years.

Since *P. lingam* data are presented as AUDPC values in the following, the infestation progress of every measured parameter is shown to visualize the relation between the absolute number and AUDPC level using the example of the untreated control at Birkenmoor.

3.1.1.1 Leaf infestation

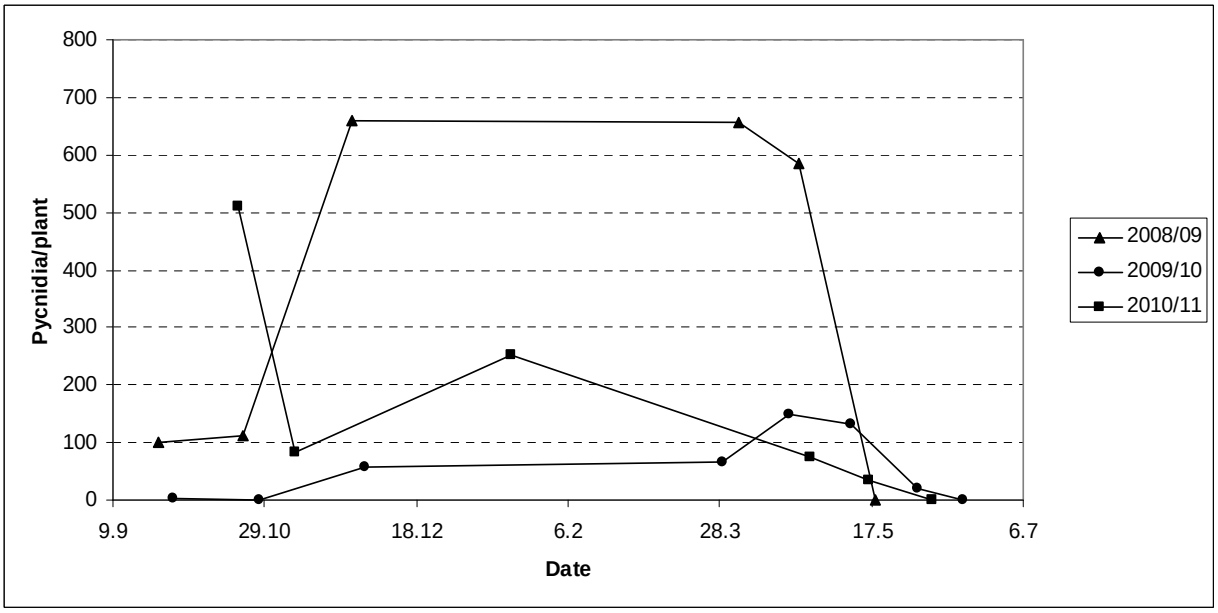


Fig. 13: Pycnidia of *Phoma lingam* per plant in the untreated control (V1) assessed at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

First symptoms of *P. lingam* always appeared in autumn, while occurrence of pycnidia was by far highest in first trial season 2008/09 (Fig. 13). The decline appeared in October 2010

was caused by the loss of leaves. In general, progress stagnated during the dormant season, while pycnidia per plant decreased at the end of spring due to the discharging of leaves.

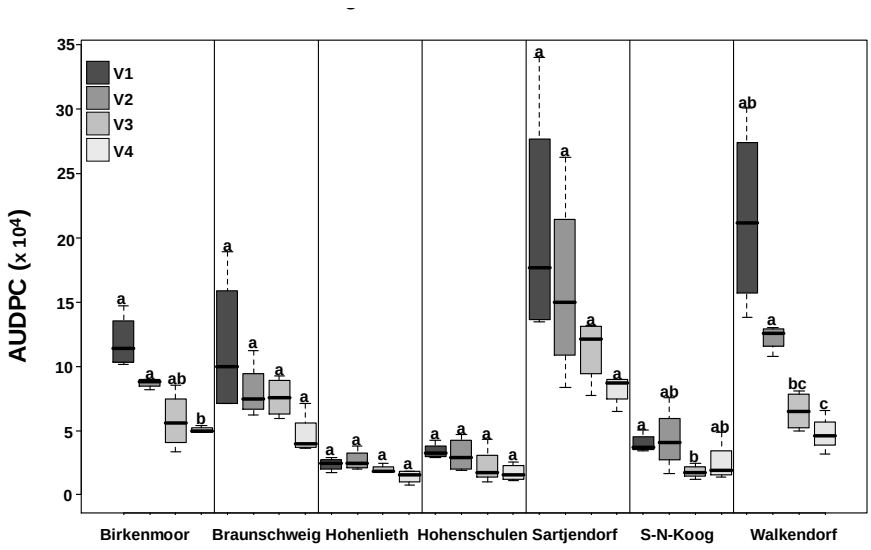


Fig. 14: *Phoma lingam* pycnidia leaf infestation expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Variation of leaf infestation was highly variable and depending on the year and site. *P. lingam* leaf infestation was highest in the first season of trials performed during 2008/09 (Fig. 14). During this season the combination of bacterial inoculation and application of fungicides (V4) reduced the pycnidia AUDPC at all sites between 38 and 78 % compared to the untreated control (V1). At Birkenmoor a significant reduction of 58 % ($T=-5.7$, $df=252$, $p<0.01$) and of 78 % in Walkendorf ($T=-4.0$, $df=252$, $p=0.012$) were recorded. V4 also reduced number of pycnidia significant compared to the single fungicide treatment V2 in Birkenmoor (42 %, $T=-13.9$, $df=252$, $p<0.01$) and Walkendorf (61 %, $T=-7.5$, $df=252$, $p<0.01$). In many cases the single bacterial treatment (V3) could achieve efficiencies that were comparable to V4. The pycnidia AUDPC in V3 was also reduced at all sites by 17-70 %, but the reduction in comparison to the control was significant only at Sönke-Nissen-Koog (55 %, $T=-4.2$, $df=252$, $p<0.01$), while the reduction compared to the single fungicide treatment V2 was significant in Walkendorf (47 %, $T=-5.4$, $df=252$, $p<0.01$). The single fungicide treatment provided a reduction of leaf pycnidia AUDPC in 5 out of 7 cases, but efficiencies were lower and non-significant.

There is a clear ranking of the variants regarding the efficiency of controlling the leaf infestation of *P. lingam* in 2008/09: V1 with the highest infestation was followed by the single treatments V2 and V3, while V4 realized the highest reduction of the AUDPC (Fig. 14).

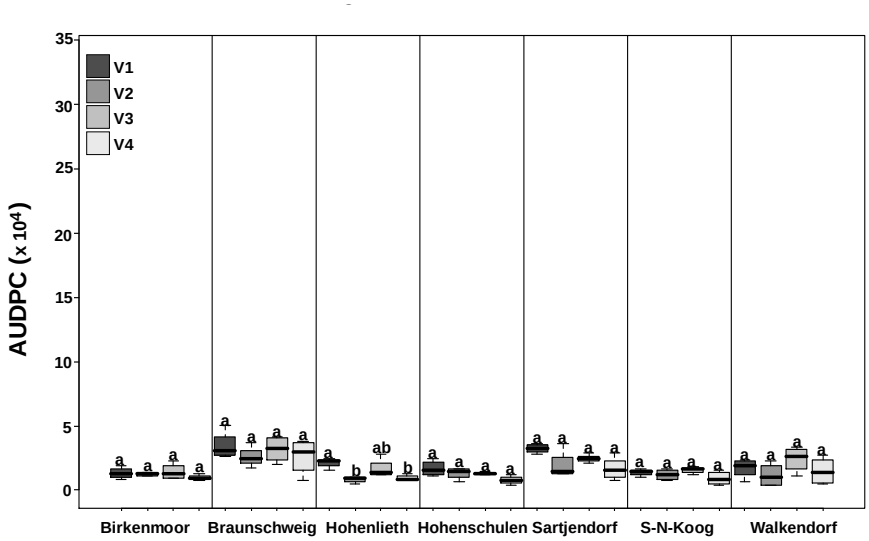


Fig. 15: *Phoma lingam* pycnidia leaf infestation expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

In the following two trial seasons the disease severity of the leaf infestation was lower and the efficiency of the single bacterial treatment and its combination with the fungicide was also lower. In 2009/10 a significant reduction of the AUDPC was recorded at Hohenlieth for V2 (62 %, T=-4.9, df=252, $p<0.01$) and V4 (56 %, T=-4.5, df=252, $p<0.01$) compared to the control while in V4 a significant reduction of the AUDPC of 63 % (T=-4.8, df=252, $p<0.01$) was assessed in Birkenmoor in 2010/11. These were the only significant differences that have been recorded during the years 2009/10 and 2010/11 (Fig. 15, Fig. 16).

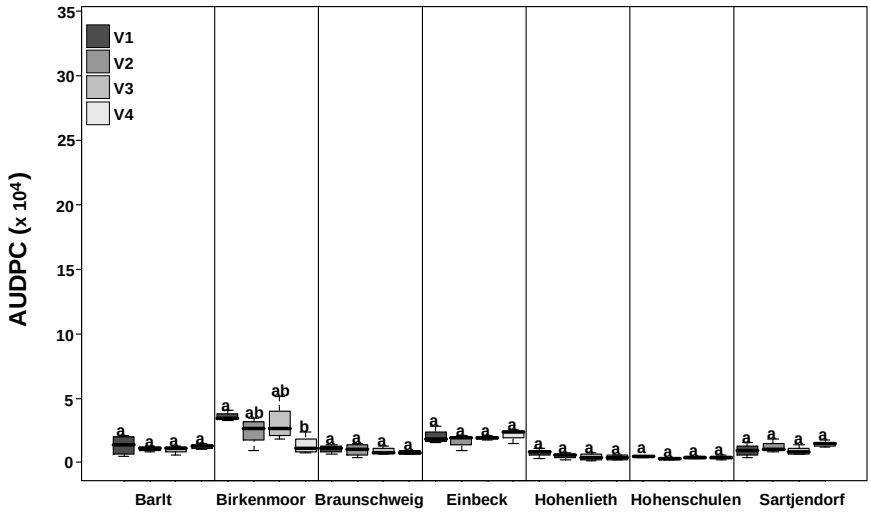


Fig. 16: *Phoma lingam* pycnidia leaf infestation expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

The frequency of infestation in the untreated control at Birkenmoor was also highest in 2008/09 (Fig. 17), while differences between the trial seasons are less expressed compared to leaf pycnidia (Fig. 13). Decreases in autumn are related to the loss of first real leaves. In 2008/09 and 2009/10 infestation frequency reached its maximum in spring and dropped to zero due to discharging of leaves end of spring/early summer.

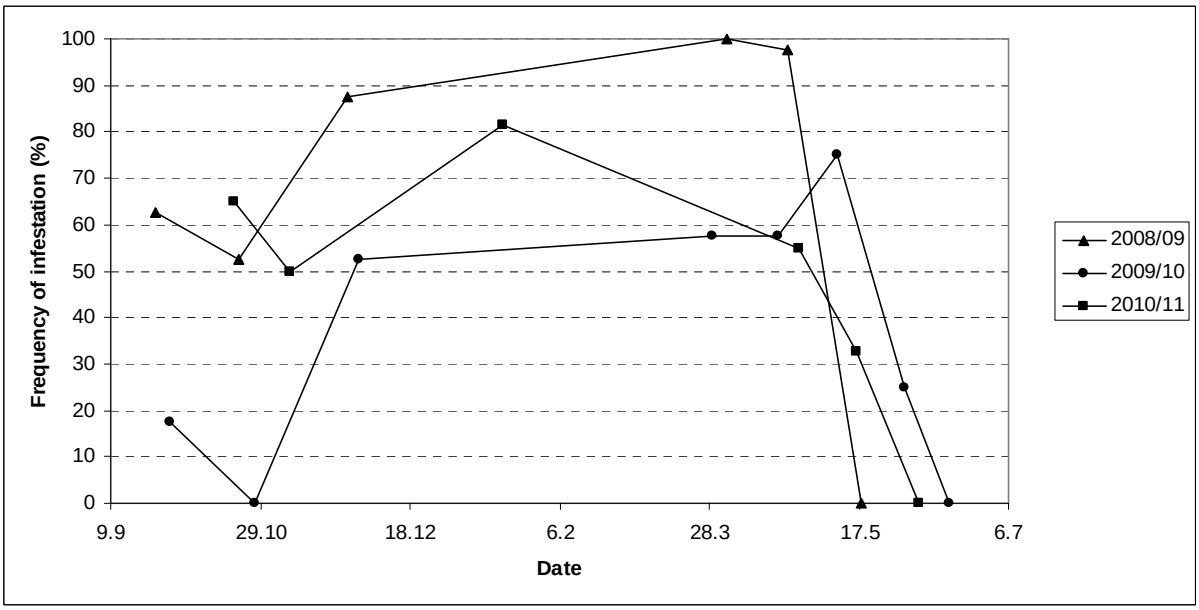


Fig. 17: Frequency of leaf infestation with *Phoma lingam* of the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

The frequency of leaf infestation was reduced in 2008/09 by the combination of bacteria and fungicides (V4) at all trial sites with differences between 11 and 26 % reduction of AUDPC when compared to the untreated control (Fig. 18).

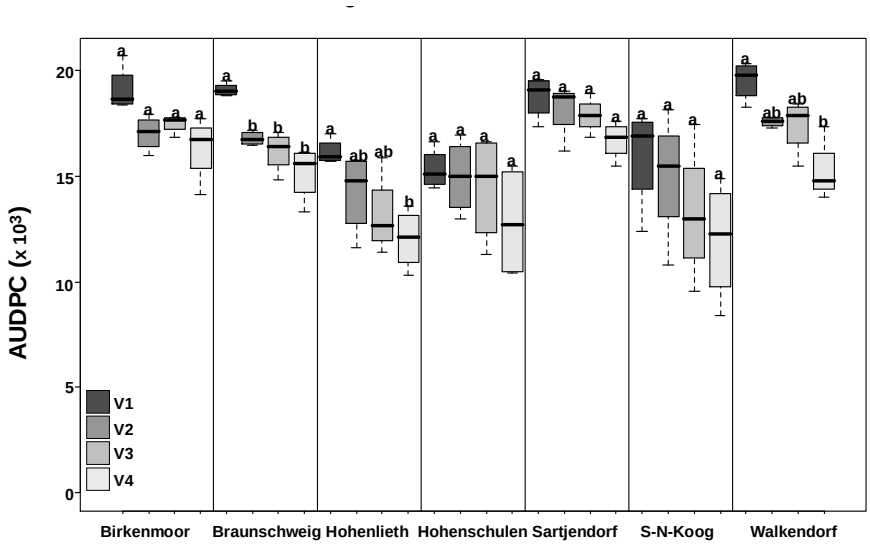


Fig. 18: Frequency of leaf infestation with *Phoma lingam* expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Effects were highly significant with a reduction of 21 % in Braunschweig (T=-5.0, df=252, p<0.001) and 26 % in Hohenlieth (T=-4.6, df=252, p<0.001) as well as 22 % in Walkendorf (T=-4.3, df=252, p=0.0028). The single bacterial and fungicidal treatments (V3; V2) also resulted in less infested plants at all sites in the first trial season (V3: 6-19 %; V2: 2-12 %), but reductions were only significant in Braunschweig (V3: 15 %, T=-5.0, df=252, p<0.001; V2: 12 %, T=-8.7, df=252, p<0.001) compared to the control (Fig. 18).

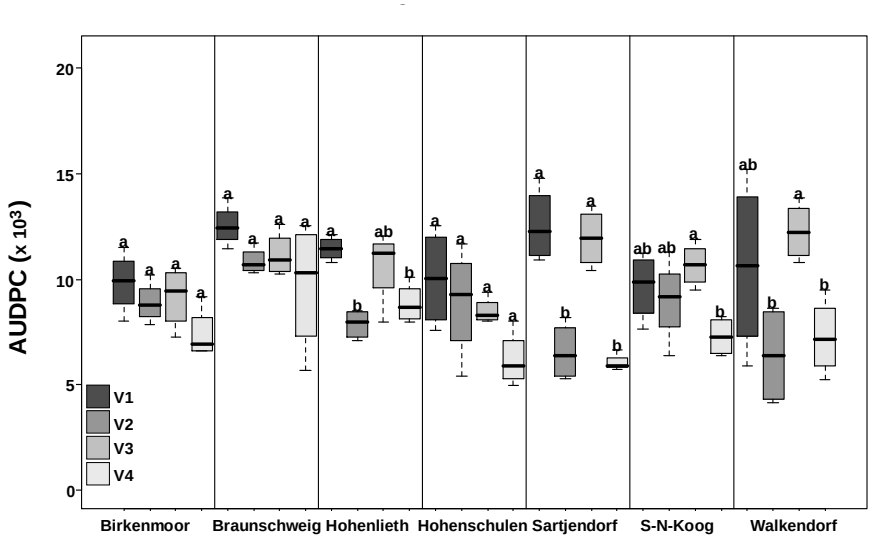


Fig. 19: Frequency of leaf infestation with *Phoma lingam* expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

In general, the frequency of infestation was lower in all variants in the following trial season 2009/10 (Fig. 19, 20). The AUDPC was not reduced by the single bacterial treatments (V3). At Sartjendorf the AUDPC for the frequency of infested plants for V3 even increased by 81 % (T=4.8, df=252, p<0.001) compared to the fungicide treatment V2 and by 97 % compared to V4 (T=7.1, df=252, p<0.001). The same effect was recorded for Walkendorf with an increase of 92 % (T=3.7, df=252, p=0.030) compared to V2 and 69 % (T=3.8, df=252, p=0.018) to V4 and at Sönke-Nissen-Koog, whereas V3 was significantly lower compared to V4 (T=4.241, df=252, p=0.0037) with an increase of 46 % of the AUDPC. Again, the strongest control effects regarding the frequency of infestation in 2009/10 was most often recorded in V4, with significant reductions in Sartjendorf (52 %, T=-6.2, df=252, p<0.001) and Hohenlieth (22 %, T=-4.1, df=252, p<0.001) compared to the control (Fig. 19).

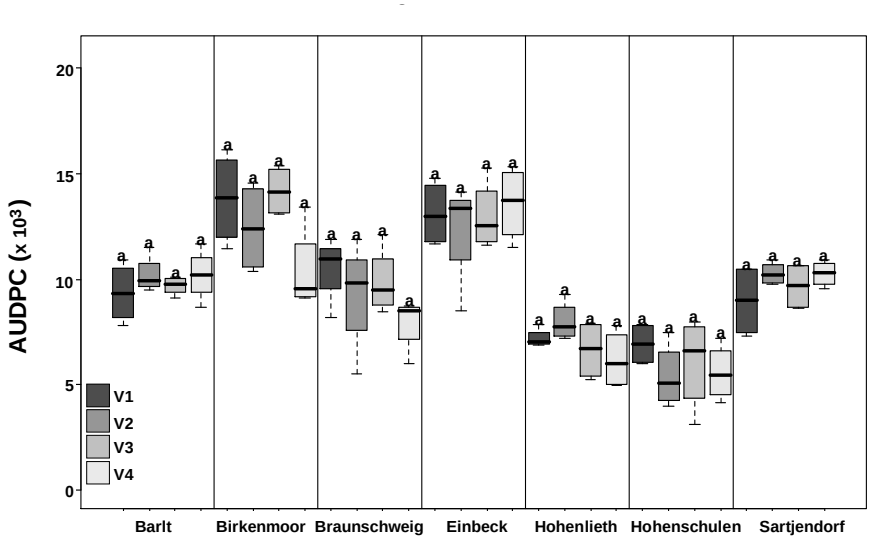


Fig. 20: Frequency of leaf infestation with *Phoma lingam* expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In the last season of trials (2010/11) no significant differences in the frequency of infestation due to treatments could be found (Fig. 20). The infestation level was comparable to the previous season (Fig. 19), while frequency of leaf infestation was highest in Birkenmoor and Einbeck. A reduction of the AUDPC was detected in Birkenmoor in V2 (10 %), whereas the combined treatment (V4) reduced the AUDPC in Birkenmoor and Braunschweig by 25 % compared to the control.

3.1.1.2 Crown canker

As shown in Fig. 21 first symptoms of crown canker appeared in May. The highest infection level was recorded during the last season of trials (2010/11). In general, the disease severity increased until harvest, but did not exceed moderate levels.

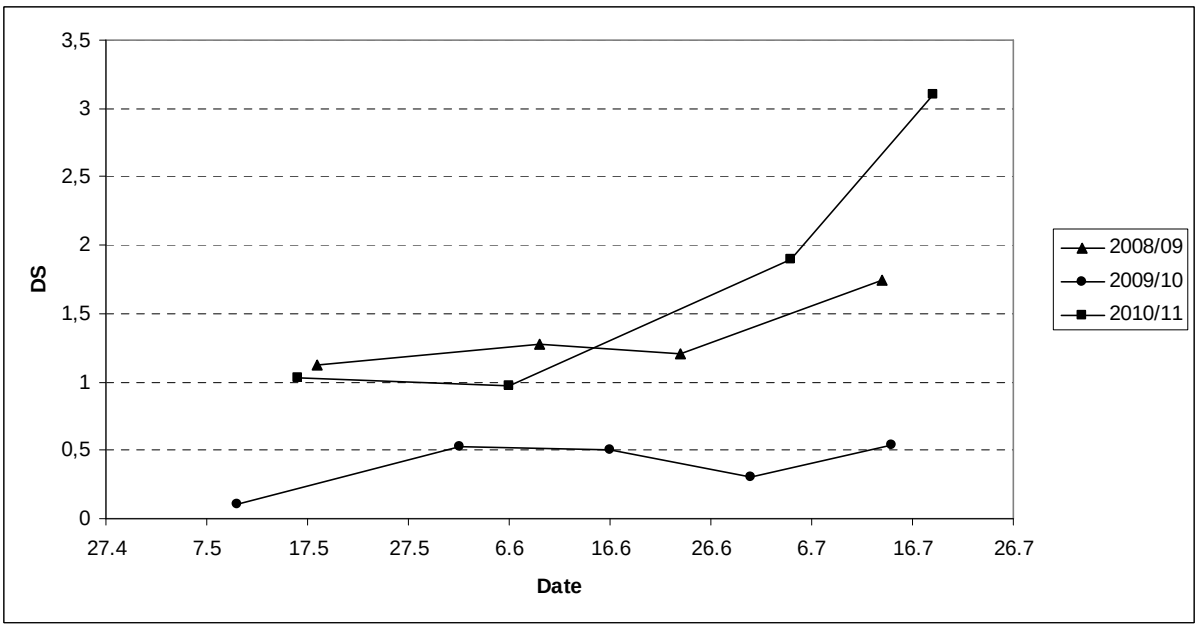


Fig. 21: Disease score (DS) of *Phoma lingam* crown canker recorded in the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

A reduction of the *P. lingam* AUDPC of crown canker symptoms in 2008/09 was recorded at 5 sites (Birkenmoor, Hohenschulen, Braunschweig, Sönke-Nissen-Koog and Sartjendorf) for all treatments (Fig. 22). The combined treatment of bacteria and fungicides led to a reduction of the AUDPC at all sites, varying between 3 and 63 %. Compared to the control, it was significant in Hohenschulen (44 %, T=-3.6, df=252, p=0.046) and Braunschweig (63 %, T=5.1, df=252, p<0.01). In average of all sites, the lowest control effect was recorded for the fungicide treatment V2. V4 had significantly lower disease scores (DS) in Hohenlieth (55 %, T=-5.4, df=252, p<0.01) and Hohenschulen (39 %, T=-3.8, df=252, p=0.0203) compared to the single fungicidal treatment. V3 reduced the AUDPC significantly in Birkenmoor compared to V1 (51 %, T= -3.8, df=252, p=0.025) and V2 (45 %, T=-3.7, df=252, p=0.033) as well as in Braunschweig (V3<V1 by 66 %, T=-7.5, df=252, p<0.01; V3<V2 by 56 %, T=-3.8, df=252, p=0.021). On the other hand, a non-significant increase of the AUDPC by 60 % was observed in Walkendorf in the single bacterial treatment in relation to the untreated control, whereas V2 could obtain a reduction of 54 % at this site.

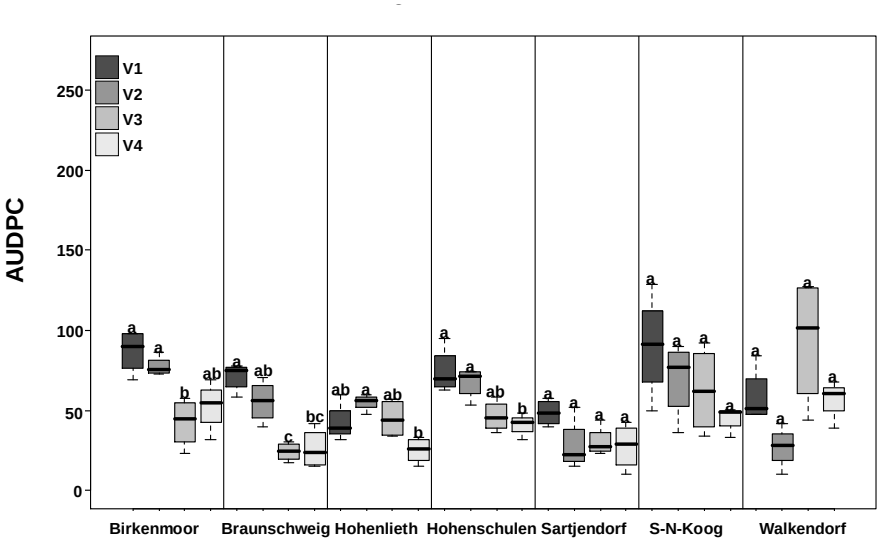


Fig. 22: Area under disease progress curve (AUDPC) of the disease score (DS) of *Phoma lingam* crown canker of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Level of crown cancer infestation was by far lowest in trial season 2009/10 and no significant differences were recorded (Fig. 23). Here infestation was most severe in Birkenmoor and Braunschweig. No important differences between the four variants were recorded.

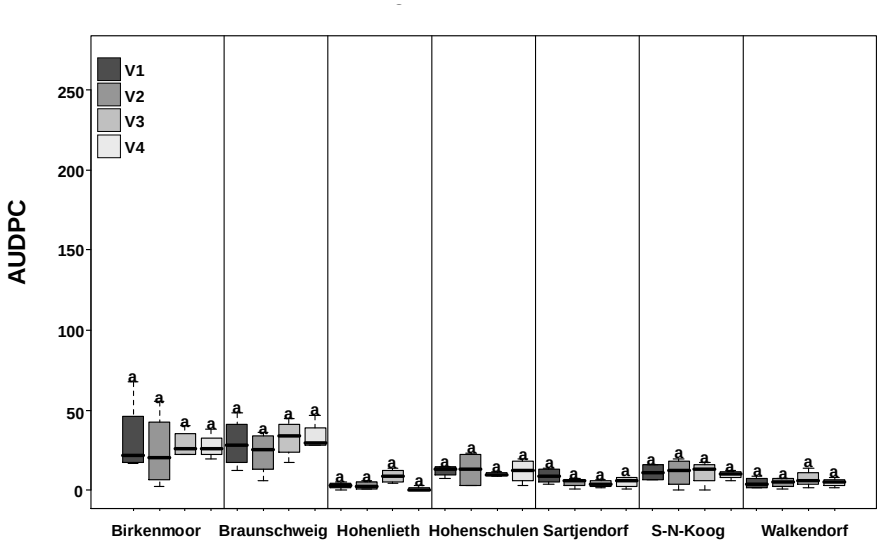


Fig. 23: Area under disease progress curve (AUDPC) of the disease score (DS) of *Phoma lingam* crown canker of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

The strongest root collar infestation was observed in the last trial season 2010/11 (Fig. 24). Contrary to 2008/09, no remarkable positive effects regarding the different treatments were assessed. Bacterial and fungicidal treatments increased the infection levels in most cases, but none of the treatments provided significant effects. The single fungicide treatment led to a higher infestation pressure in 6 out of 7 cases, including an increase of 35 % of the crown cankers AUDPC in Braunschweig. The bacterial treated variants V3 and V4 revealed increased infestations at three sites. V3 could reduce the AUDPC in Barlt and Sartjendorf by almost 20 %, whereas the combined treatment increased the AUDPC in Braunschweig by 44 %.

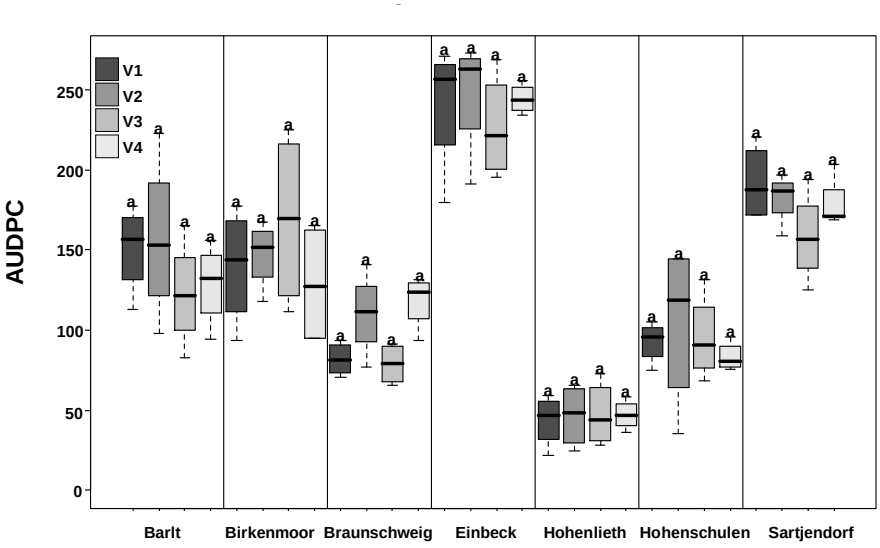


Fig. 24: Area under disease progress curve (AUDPC) of the disease score (DS) of *Phoma lingam* crown canker of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

The level of crown canker pycnidia was comparatively low in relation to leaf infestation (Fig. 25). Total amount of pycnidia increased until harvest, whereas occurrence was highest in 2008/09 and 2010/11.

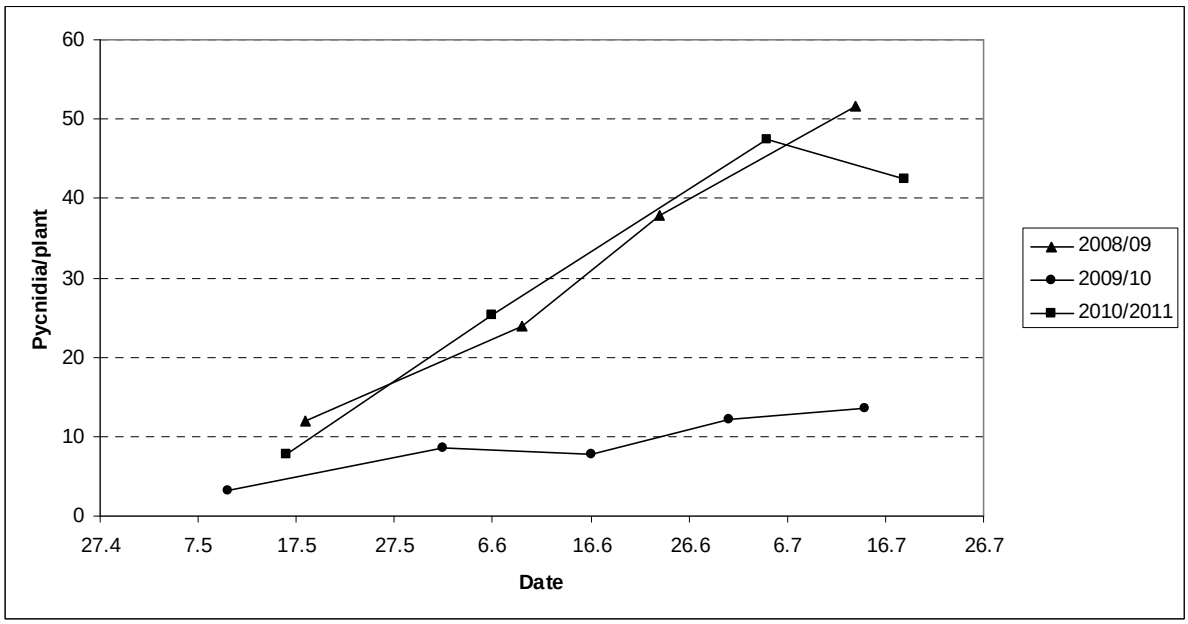


Fig. 25: *Phoma lingam* crown canker pycnidia recorded in the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

The single fungicide treatment (V2) reduced crown canker pycnidia at 5 out of 7 sites in 2008/09 (Fig. 26). Compared to controls, the AUDPC of V2 was reduced by 50 % in Sartjendorf and 66 % in Walkendorf due to the fungicide treatment, although the data were not significantly different. However, pycnidia were significantly reduced in V2 compared to V4 (60 %, T=-5.9, df=252, p<0.01) in Walkendorf. V3 provided high efficiencies at all sites except for Hohenlieth and Walkendorf. Reductions due to bacterial treatments (V3) were significant in Birkenmoor compared to V1 and V2 (3<1: 59 %, T=-3.6, df=252, p=0.044; 3<2: 60 %, T=-5.4, df=252, p<0.01) and in Braunschweig compared to V1 (68 %, T=-4.4, df=252, p<0.01). In 2008/09 crown canker pycnidia were reduced by the combination of bacteria and fungicides (V4) at all trial sites, while efficiencies differed between 16 and 73 % reduction of the AUDPC compared to the control. Reduction was significant in Braunschweig (4<1: 73 %, T=-5.0, df=252, p<0.01) and almost significant at Sönke-Nissen-Koog (4<1: 62 %, T=-3.4, df=252, p= 0.083).

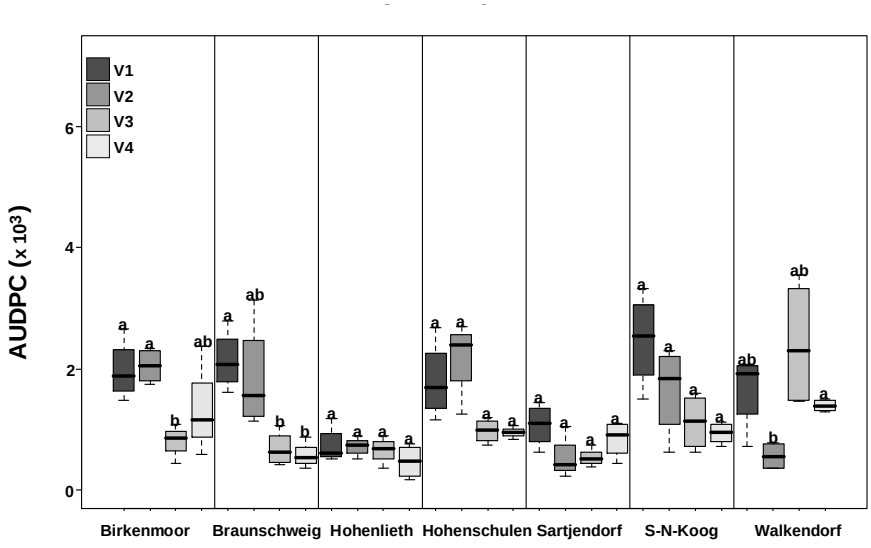


Fig. 26: *Phoma lingam* crown canker pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Occurrence of crown canker pycnidia in 2009/2010 was the lowest within the period of investigation (Fig. 27). Analogous to the disease score, the number of pycnidia was highest

in Birkenmoor and Braunschweig. No differences between the variants were determined during this trial year.

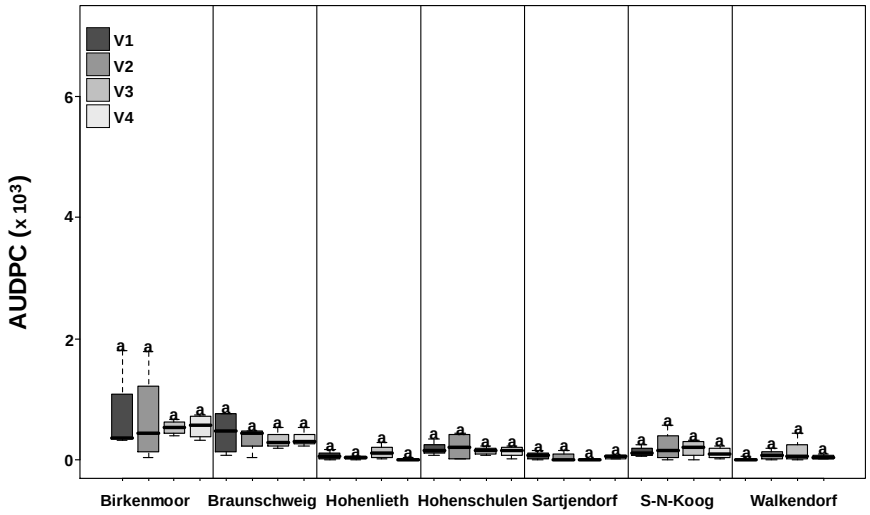


Fig. 27: *Phoma lingam* crown canker pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In the last trial season 2010/11 treatments led to increased AUDPC for number of crown canker pycnidia in most cases (Fig. 28). V2 and V4 had significant more pycnidia at root collars in Braunschweig (V2<V1: 103 %, T=5.4, df=252, p<0.01; V4<V1: 153 %, T=5.6, df=252, p<0.01), while the AUDPC of V4 was significant higher in Sartjendorf (34 %, T=4.4, df=252, p<0.01) compared to V3. Fungicide treated variants led to non-significant increases of 103 (V2) and 154 % (V4) in Braunschweig as well as 168 (V2) and 198 % (V4) in Einbeck. Barlt was the only site in 2010/11 where treatments had a positive effect. Here the single bacterial treatment could reduce the pycnidia AUDPC by 37 % compared to the control, which was non-significant. Due to wet soil conditions there was no application of fungicide in autumn in Barlt.

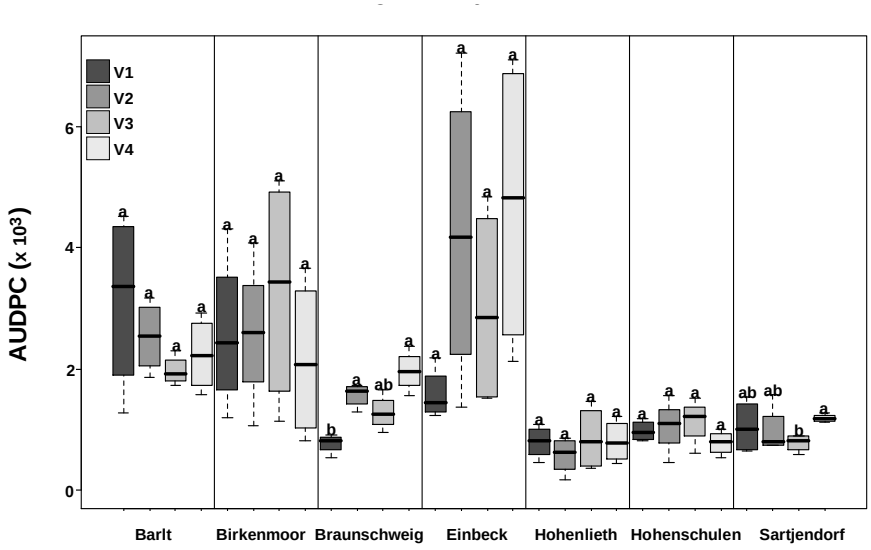


Fig. 28: *Phoma lingam* crown canker pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Crown canker frequencies reflect the progresses of disease score and pycnidia (Fig. 29). Infestation frequency was highest in 2010/11, followed by trial season 2008/09.

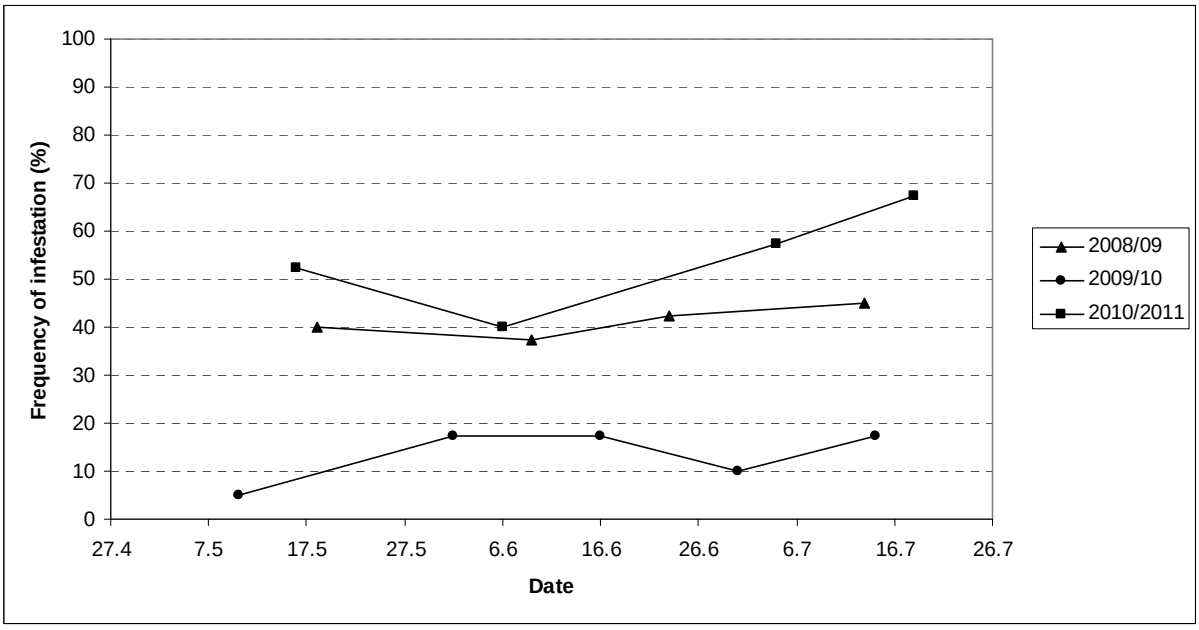


Fig. 29: Frequency of *Phoma lingam* crown canker of the untreated control (V1) at Birkenmoor recorded during the trial years 2008/09, 2009/10, 2010/11.

The combination of bacteria and fungicides (V4) could reduce the frequency of crown canker at all trial sites in 2008/09, while efficiencies differed between 7 and 59 % reduction of the AUDPC compared to the control (Fig. 30). Reductions were significant in Braunschweig (4<1: 59 %; T=-4.0, df=252, p=0.013; 4<2: 50 %, T=-3.6, df=252, p=0.039), Birkenmoor (4<1: 38 %; T=-4.2, df=252, p<0.001; 4<2: 31 %, T=-4.1, df=252, p<0.001) and almost significant in Hohenschulen (4<1: 42 %; T=-3.4, df=252, p=0.0723). The single bacterial and fungicidal treatments (V3; V2) led to reductions of infestation frequency at 6 out of 7 sites in the first season of trials. While V2 reached non-significant effects of up to 42 %, significant reductions were found in V3 in Braunschweig (3<1: 58 %, T=-4.5, df=252, p<0.01; 3<2: 48 %, T=-4.8, df=252, p<0.01) and Hohenschulen (3<1: 37 %, T=-4.6, df=252, p<0.01). On the other hand, V3 provided a 46 % higher AUDPC in Walkendorf compared to the control.

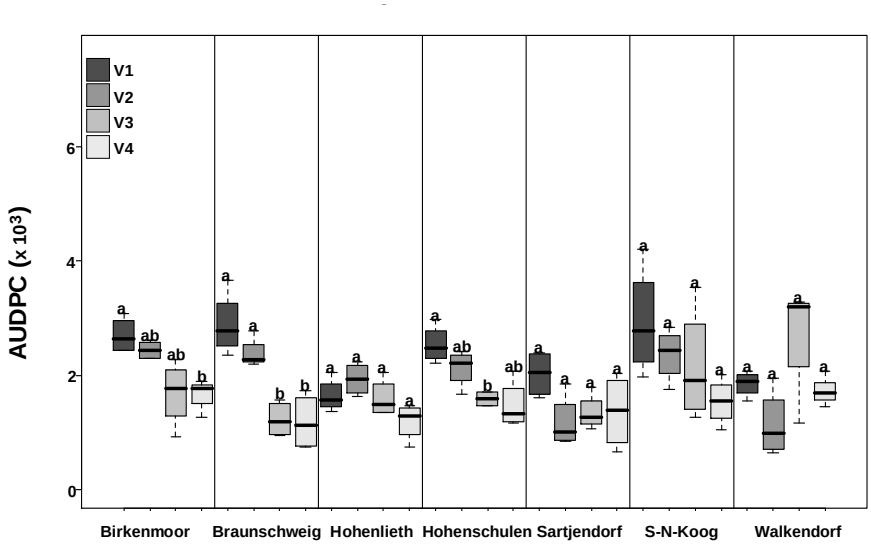


Fig. 30: Frequency of *Phoma lingam* crown canker expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Besides DS and pycnidia, frequency of infestation was also lowest in trial season 2009/10. AUDPC were highest again in Birkenmoor and Braunschweig, while no important differences were recorded (Fig. 31).

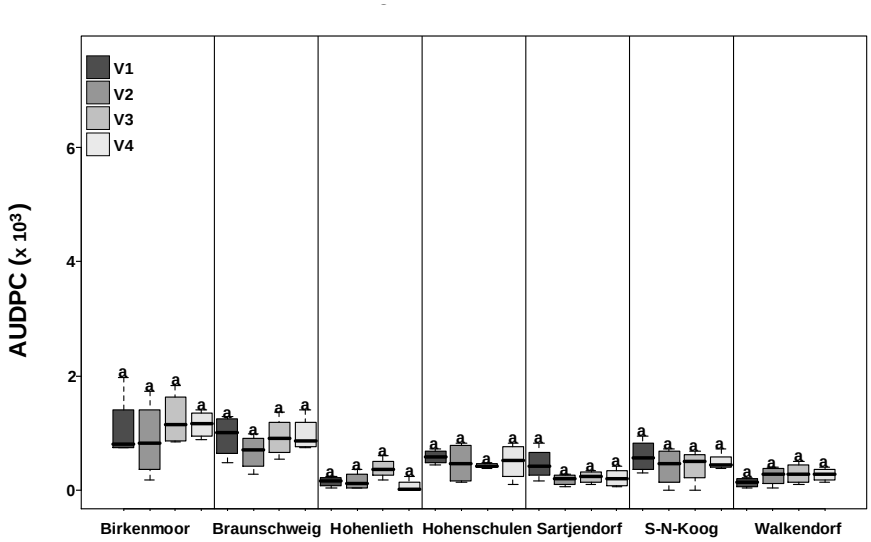


Fig. 31: Frequency of *Phoma lingam* crown canker expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In contrast to DS and pycnidia there were no significant differences regarding the frequency of infestation in the last season of trials. The frequency was by far highest in Einbeck, whereas no differences between variants were recorded at that site (Fig. 32). In Braunschweig, AUDPC of fungicide treated variants V2 and V4 were increased by 27 and 33 % compared to the control, while all treatments reduced the frequency of crown cancer in Sartjendorf up to 15 % (V4).

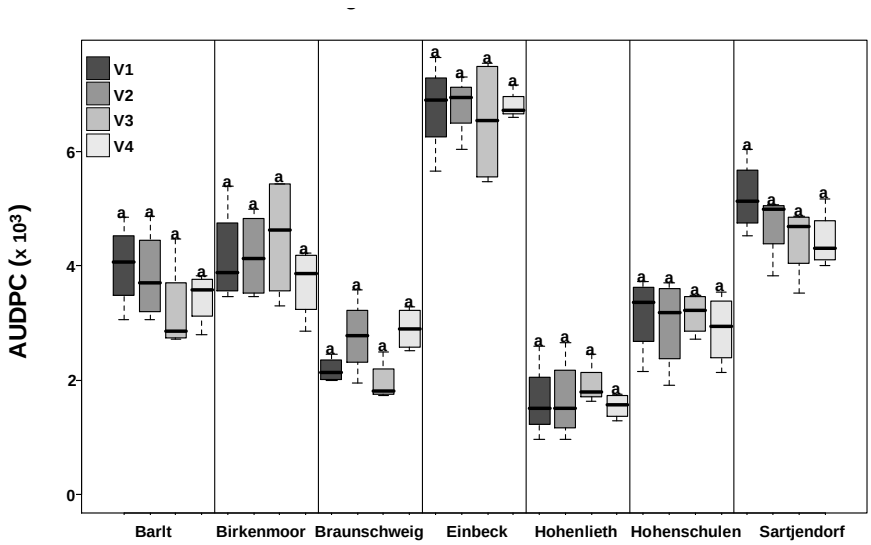


Fig. 32: Frequency of *Phoma lingam* crown canker expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

3.1.1.3 Stem infestation

Data of *P. lingam* stem disease score revealed a significant influence of the variant (V) ($F=4.0$, $df=3, 252$, $p=0.0083$) and site and year (SY) ($F=110.2$, $df=20, 252$, $p<2.2 \log_{10}-16$) and no significant interaction between SY and V.

As shown in Fig. 33 first symptoms of stem infestation also appeared in May and did not evolve until the beginning of July. Serious infestation only took place in July 2009 and 2011.

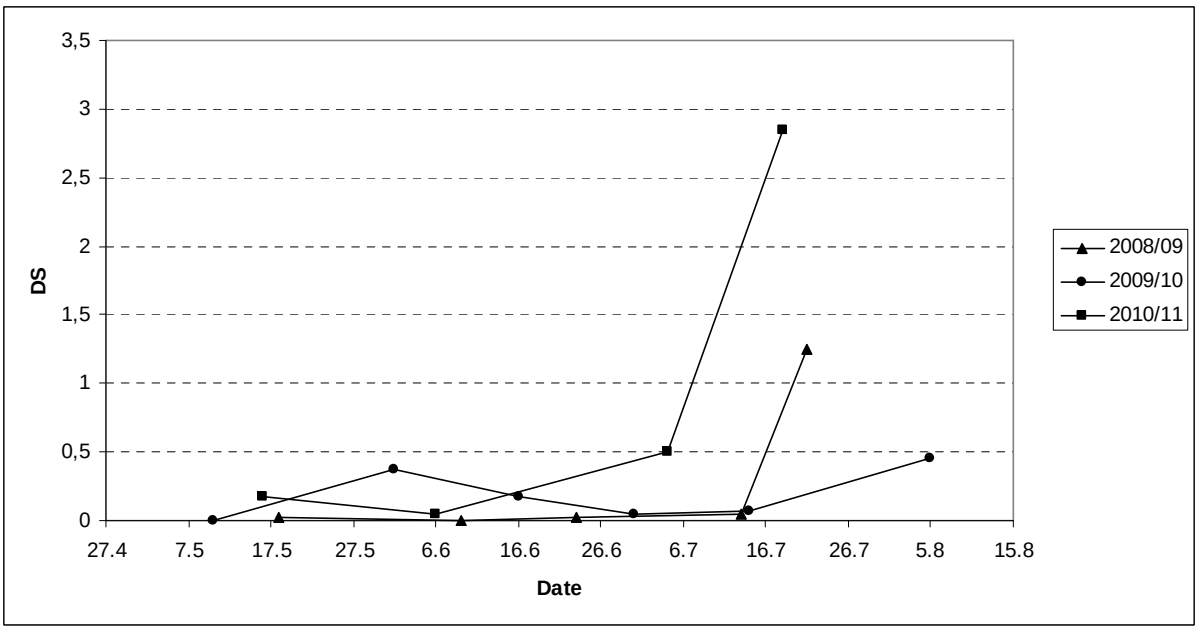


Fig. 33: *Phoma lingam* stem infestation of the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

Stem infestation was less severe than leaf infestation and crown canker during the period of investigation and there were no significant differences between the variants.

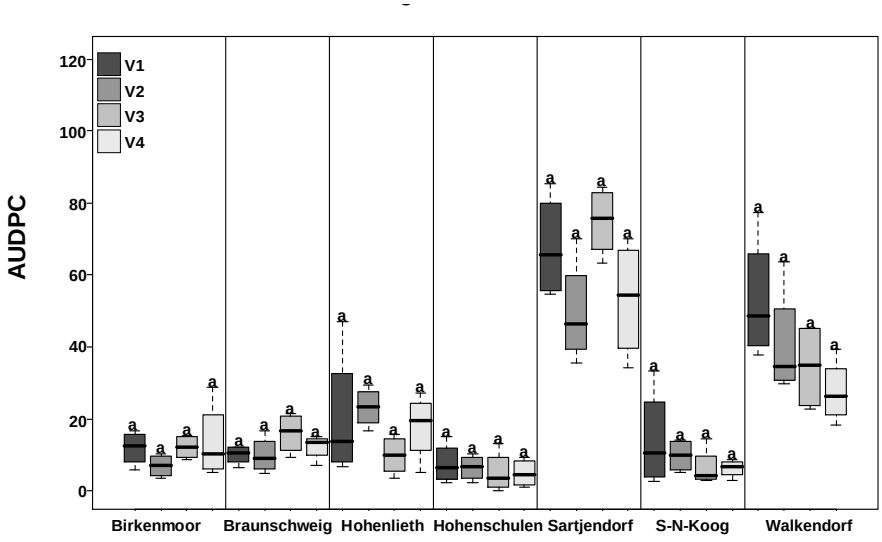


Fig. 34: *Phoma lingam* disease score (DS) of stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

During the first season of trials mentionable stem infestation did occur in Sartjendorf and Walkendorf (Fig. 34). In Sartjendorf the fungicide treatment reduced the AUDPC (V2-V1: 27 %; V4-V1: 21 %) while the single bacterial treatment increased the AUDPC by 10 % compared to the control. However, all treatments in Walkendorf led to decreased infestation, whereas the effect was highest in V4 (48 %).

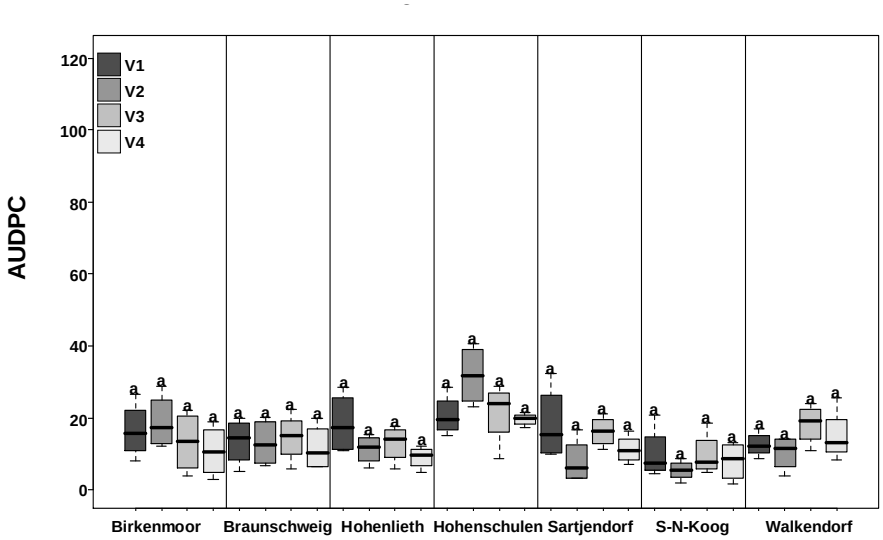


Fig. 35: *Phoma lingam* disease score (DS) of stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In 2009/10 severity of stem DS was lowest within the investigation period. Infestation was highest in Hohenschulen (Fig. 35). The fungicide treatment led to an increased AUDPC (V2-V1: 54 %) while other variants did not differ from the control. In contrast to Hohenschulen, in fungicide treated variants a decrease of the AUDPC was recorded in Sartjendorf by 56 % (V2-V1) and 38 % (V4-V1). In absolute terms, all differences between variants are negligible due to the low infestation intensity in this year.

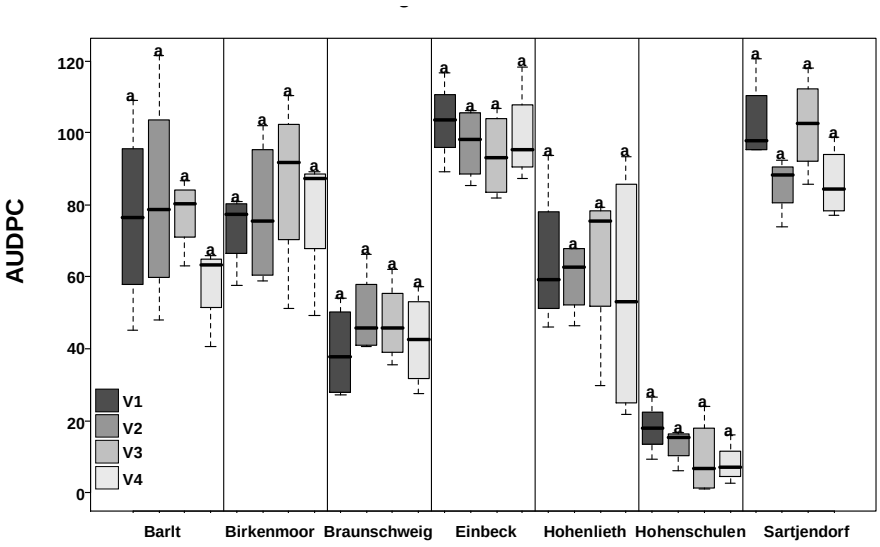


Fig. 36: *Phoma lingam* disease score (DS) of stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In the last season of trials stem infestation was –except for Hohenschulen- considerably increased compared to previous years (Fig. 36). Infestation was strongest in Barlt, Birkenmoor, Einbeck and Sartjendorf. The combined treatment decreased the AUDPC by 24 % (V4-V1) in Barlt, while both fungicide treatments led to a reduction of the AUDPC in Sartjendorf (V2-V1: 13 %; V4-V1: 6 %). On the other hand, V4 and V3 led to an increased AUDPC in Birkenmoor (V4-V1: 7 %; V3-V1: 18 %). Despite the higher infestation level, no significant differences between the variants occurred.

The level of crown stem pycnidia also was comparatively low compared to leaf infestation (Fig. 37). While occurrence was hardly measurable in the beginning, amount of pycnidia explosively increased before harvest. Severity was highest in 2008/09 and 2010/11.

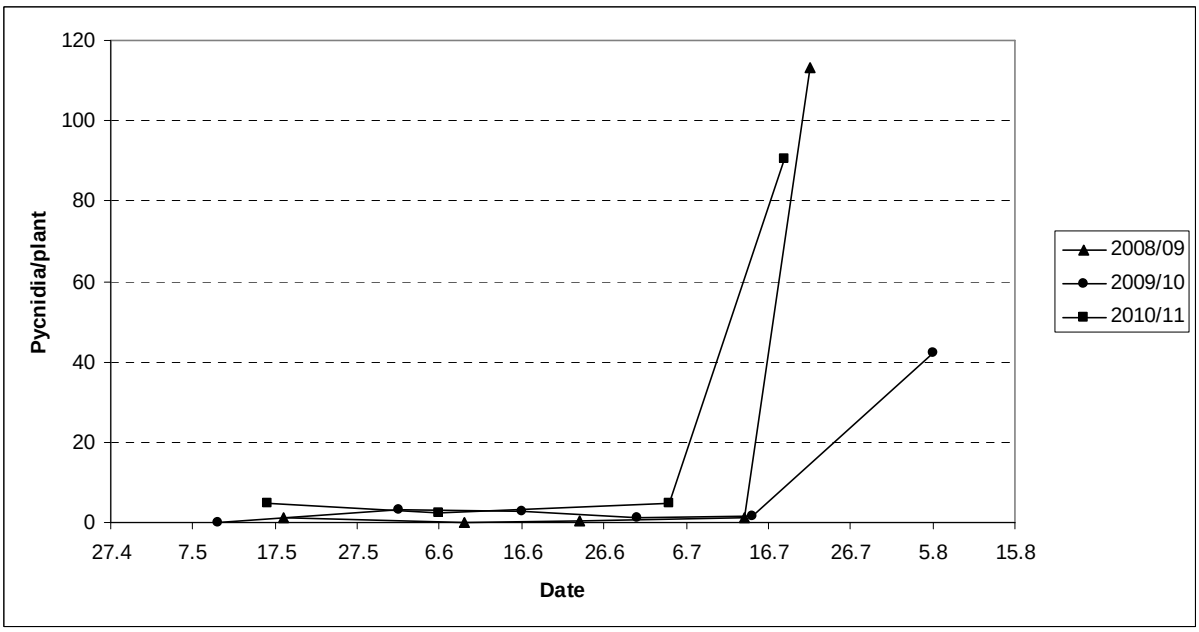


Fig. 37: *Phoma lingam* stem pycnidia of the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

Data of *P. lingam* stem pycnidia revealed a highly significant interaction between SY and V ($F=2.5$, $df=60, 252$, $p=6.3 \log_{10} -07$).

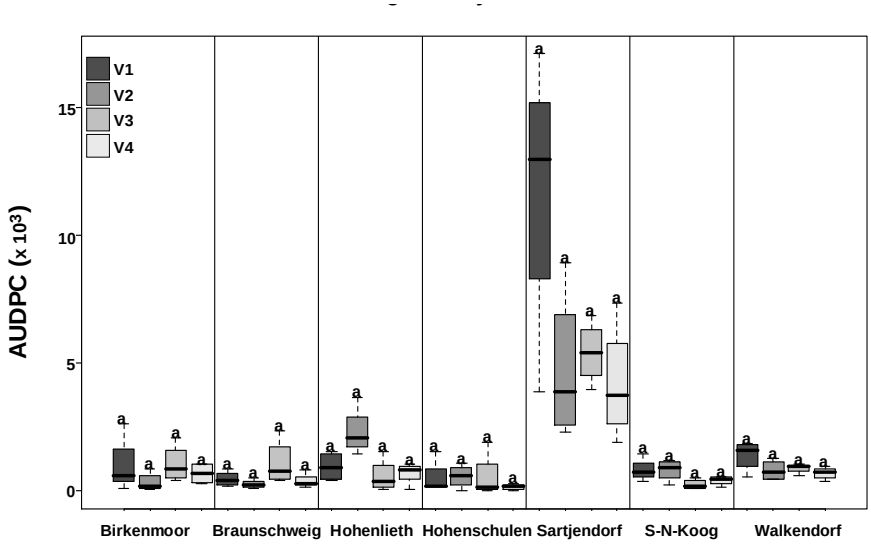


Fig. 38: *Phoma lingam* stem pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

Only in Sartjendorf occurrence of stem pycnidia was severe in 2008/09 (Fig. 38). Here all treatments could realize a reduction that ranged between 54 % (V3-V1) and 64 % (V4-V1). Furthermore, the single fungicide treatment led to an increased infestation in Hohenlieth.

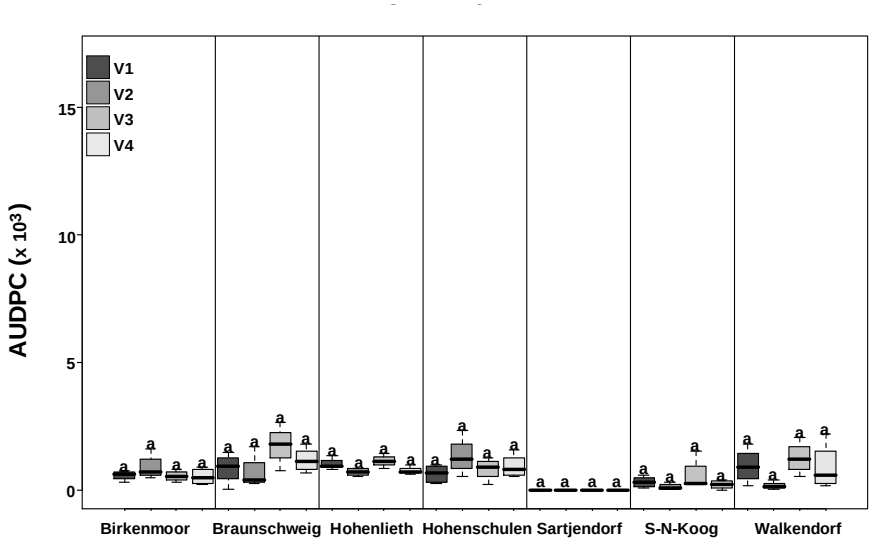


Fig. 39: *Phoma lingam* stem pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In 2009/10 no relevant infestation with pycnidia appeared. Infestation in V3 was increased in Braunschweig compared to the control, while V2 led to a higher AUDPC in Hohenschulen (Fig. 39). In Walkendorf a reduction of almost 80 % (V2-V1) was provided through the fungicide application (V2), but infestation level was very low.

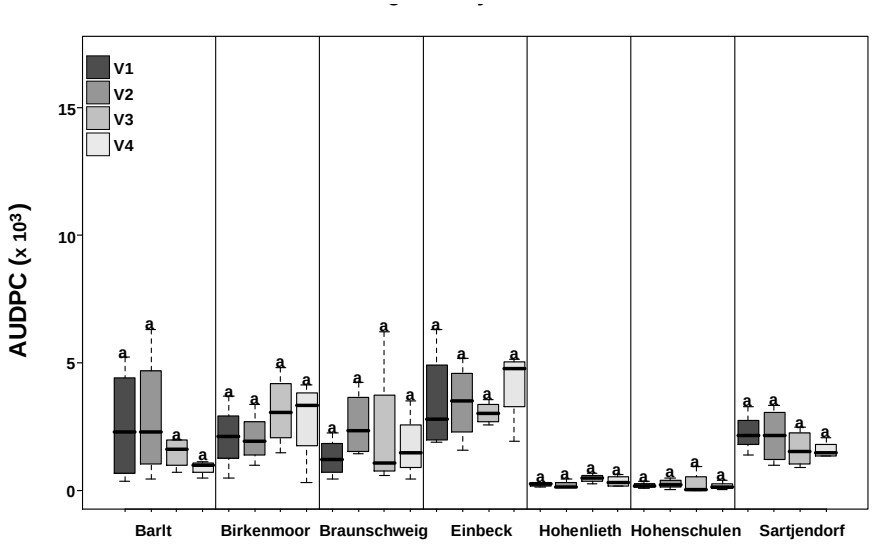


Fig. 40: *Phoma lingam* stem pycnidia expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

Analogous to the disease index, pycnidia infestation level was also highest in 2010/11 (Fig. 40). Most affected were Barlt, Birkenmoor, Braunschweig and Einbeck. Except for Barlt, treatments had no influence on the amount of pycnidia. In Barlt AUDPC in bacterial treated variants was reduced by 41 % (V3) and 64 % (V4) compared to the control, while all treatment led to increased AUDPC in Einbeck.

Stem infestation frequencies of the untreated control at Birkenmoor are presented in Fig. 41. Infestation frequency increased in July in all three trial seasons and was highest in 2010/11, followed by trial season 2008/09.

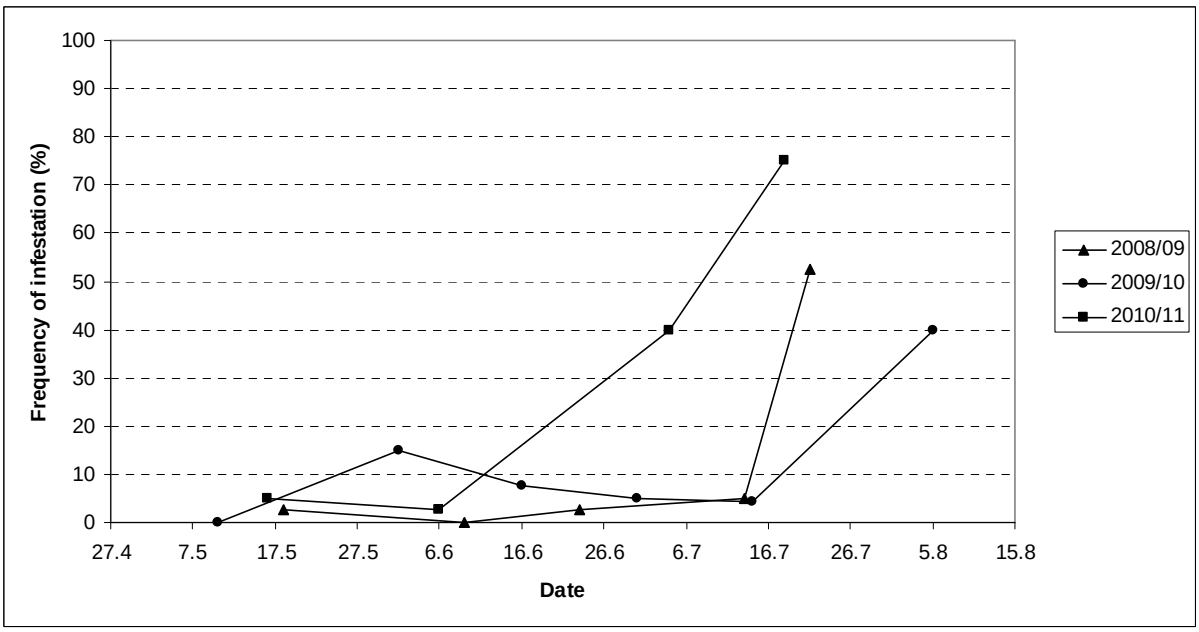


Fig. 41: Frequency of *Phoma lingam* stem infestation of the untreated control (V1) at Birkenmoor during the trial years 2008/09, 2009/10, 2010/11.

Data of *P. lingam* stem infestation frequency revealed that V ($F=3.6$, $df=3, 252$, $p=0.014$) and SY ($F=77.8$, $df=20, 252$, $p< 2.2 \log_{10} -16$) had a significant influence while there is no significant interaction between SY and V.

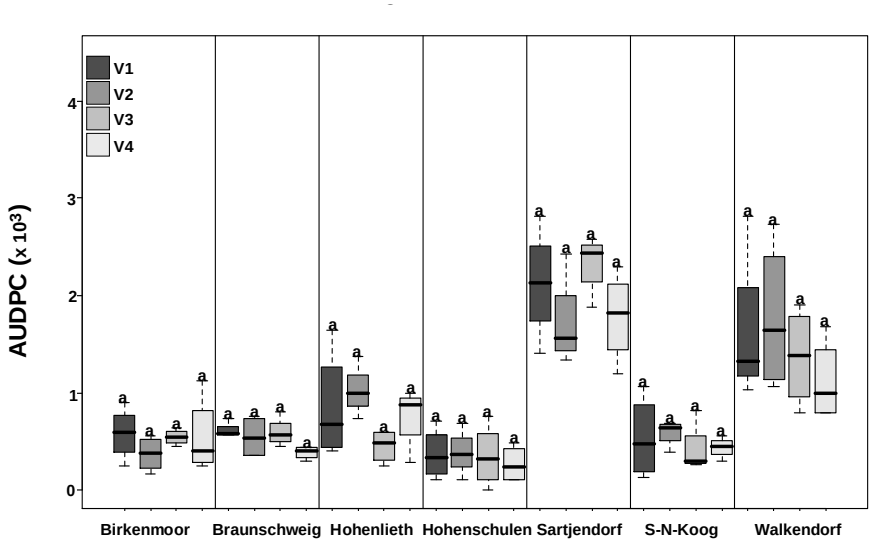


Fig. 42: Frequency of *Phoma lingam* stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

Compared to the level of leaf infestation and crown canker, *P. lingam* stem infestation was less pronounced throughout this investigation period. In 2008/09 infestation was highest in Sartjendorf and Walkendorf, while V4 led to a reduction of AUDPC at both sites (Sartjendorf V4-V1: 16 %; Walkendorf V4-V1: 31 %) (Fig. 42). In Sartjendorf the single fungicidal treatment V2 reduced the AUDPC by 19 % (V2-V1), whereas the single bacterial treatment V3 led to increased AUDPC in Sartjendorf (V3-V1: 10 %) and Walkendorf (V3-V1: 25 %)

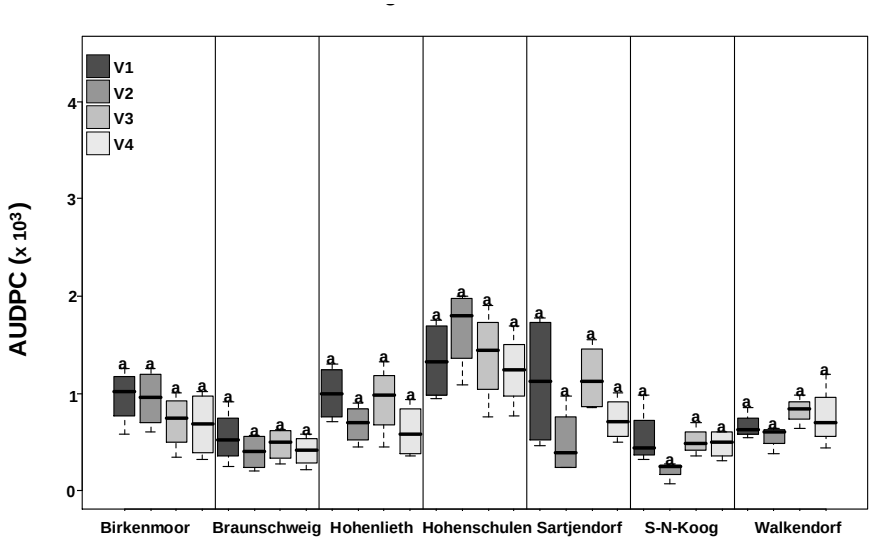


Fig. 43: Frequency of *Phoma lingam* stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In 2009/10 frequency of stem infestation was highest in Hohenschulen and Sartjendorf. The fungicide treated variants V2 and V4 realized again reductions of AUDPC of 56 % (V2-V1) and 35 % (V4-V1). Except for Hohenschulen V2 reduced the frequency of infestation at every site in this season, whereas the single bacterial treatment was at the same level as the control in most cases (Fig. 43).

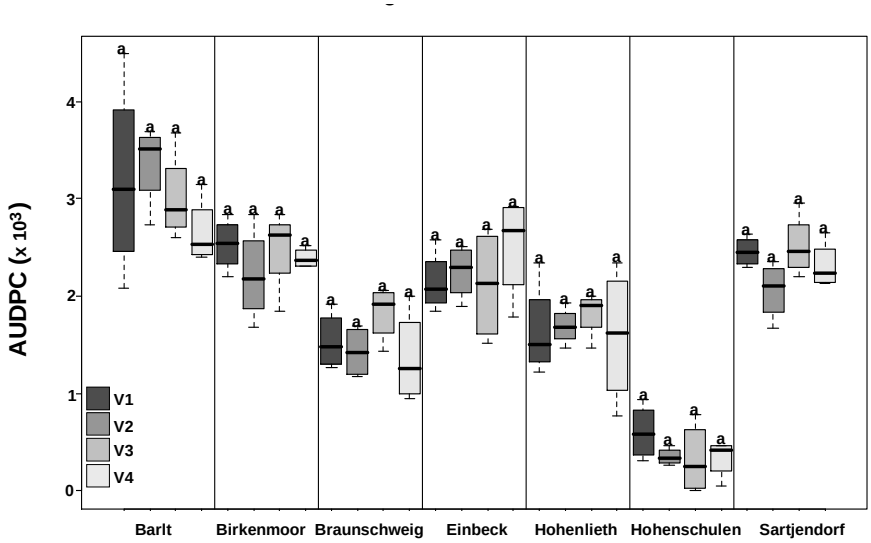


Fig. 44: Frequency of *Phoma lingam* stem infestation expressed as AUDPC (area under disease progress curve) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. AUDPC values are presented as box plots. Box plots within one frame followed the same different letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

Level of infestation frequency was highest in the last season of trials (Fig. 44). Most infested plants were found in Barlt, Birkenmoor, Einbeck and Sartjendorf. In Barlt the AUDPC of V4 was decreased by 19 % compared to the control, while combined treatment increased the AUDPC by 17 % in Einbeck. The single fungicidal treatment could slightly reduce AUDPC in Birkenmoor (12 %) and Sartjendorf (16 %), while other variants did not noticeably differ at these sites.

3.1.1.4 Regression analyses of ***Phoma lingam*** parameters

Regression analysis related leaf pycnidia with root collar pycnidia (slope: 7.56×10^{-4}, T=0.34, df=168, p=0.63) and crown canker disease score (slope: 7.55×10^{-5}, T=1.13, df=168, p=0.26) showed a very small positive relation that is not significant. Putting frequency of infestation of leaves and root collars in relation resulted in a non-significant negative relation (slope: -6.37×10^{-3}, T=-0.33, df=168, p=0.74).

3.1.2 *Verticillium longisporum*

Regarding to disease score data for the infestation with *V. longisporum*, the combined variable of site and year (SY) and the variant (V) had a significant influence (SY: F=96.0, df=16, 204, p<2.2 $\log_{10}$ -16; V: F=4.6, df=3, 204, p=0.0037). There are no significant interactions between SY and V. Sites showing no infestation were excluded from statistical analysis.

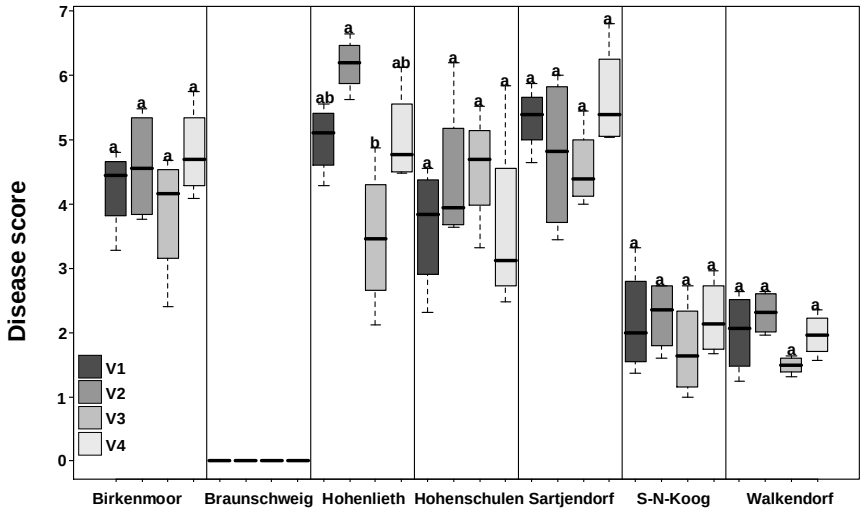


Fig. 45: *Verticillium longisporum* disease score (DS) of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. DS are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

V. longisporum infestation was highest in the first season of trials (2008/09). The single-fungicidal treatment V2 led to a higher infestation pressure in 6 out of 7 cases including a highly significant (T=5.6, df= 204, p<0.001) increase by 77 % compared to V3 (Fig. 45). Reduction of the disease score through the single-bacterial treatment V3 was also worth mentioning, albeit not significant in Walkendorf (3<1: 26 %; 3<2: 35 %; 3<4: 24 %) and Sartjendorf (3<1: 15 %). Other than at Hohenschulen, where V3 showed the highest DS with 4.56, the variant V3 revealed lowest DS in this trial season at all sites. In most cases the DS of the combined treatment of bacteria and fungicides V4 was at the same level as the untreated control.

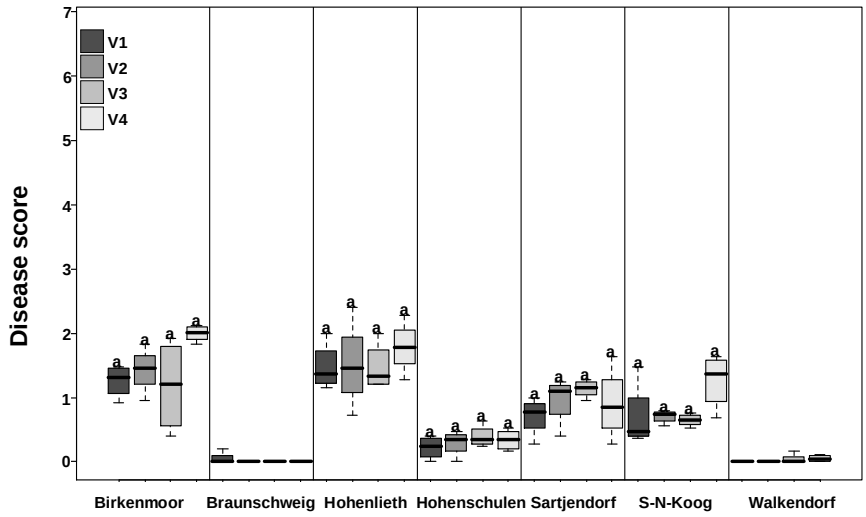


Fig. 46: *Verticillium longisporum* disease score (DS) of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. DS are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

During the following trial season 2009/10, *V. longisporum* infestation was, in general, lower in all variants. No significant differences were recorded between the treatments. There was noticeable emergence of the disease at Birkenmoor, Hohenlieth, Sartjendorf and Sönke-Nissen-Koog (Fig. 46). At all presented sites, V4 had the highest DS (Birkenmoor: 1<4: 59 %; Hohenlieth: 1<4: 21 %; Sönke-Nissen-Koog: 1<4: 80 %), and none of the treatments realized any control effect.

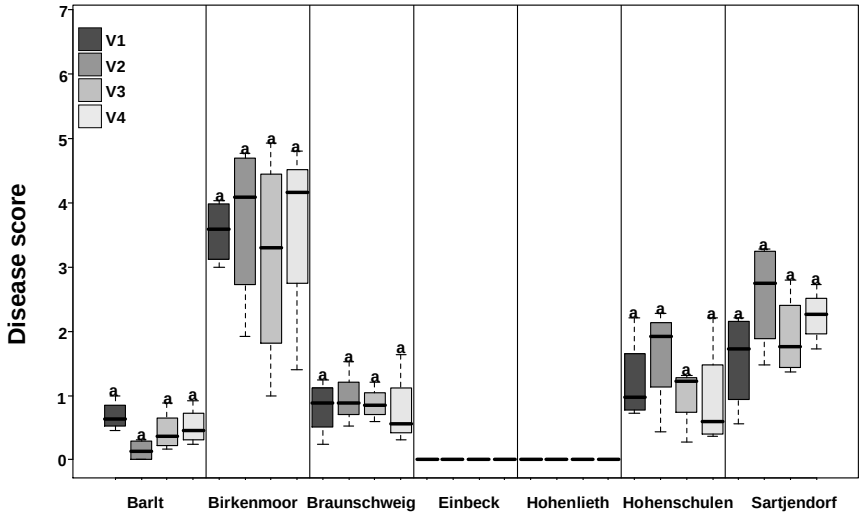


Fig. 47: *Verticillium longisporum* disease score (DS) of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. DS are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

During the last investigation period, severity increased again especially in Birkenmoor (Fig. 47). At that site, the infestation in fungicide-treated variants V2 and V4 was increased again, while V3 realized a reduction of the DS by 12 % compared to control. Again V2 revealed highest DS at all sites with stronger infestation (Birkenmoor: 1<2: 5 %; Hohenschulen: 1<2: 34 %; Sartjendorf: 1<2: 65 %). On the other hand the single fungicide treatment showed the lowest DS in Barlt but infestation level was low too.

Regarding to the frequency of infestation data for the infestation with *V. longisporum*, the combined variable of site and year had a significant effect ($F=61.7$, $df=16, 204$, $p<2\ \log_{10}\text{-}16$). There were no significant interactions between SY and V.

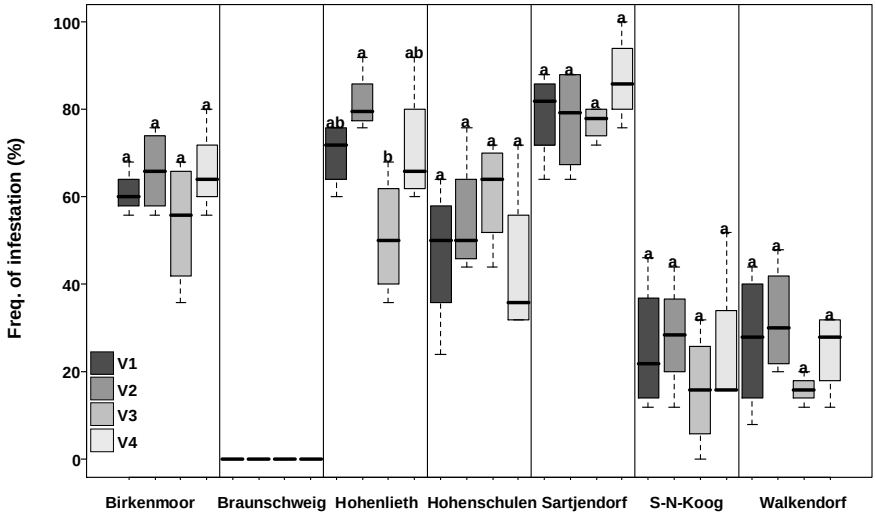


Fig. 48: *Verticillium longisporum* frequency of infestation of field trials in 2008/09. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. Values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

V. longisporum was detected at 6 out of 7 sites in 2008/09. In Birkenmoor, Hohenlieth and Sartjendorf clearly more than 50 % of the plants were infested by *V. longisporum* (Fig. 48). Relations between variants almost correspond with the DS data in this season. In Hohenlieth frequency of infestation was reduced significantly by V3 compared to the single fungicide treatment (3<2: 60 %, p=0.028). Further V3 showed less infected plants at all sites except for Hohenschulen, where the combined treatment V4 was least infected.

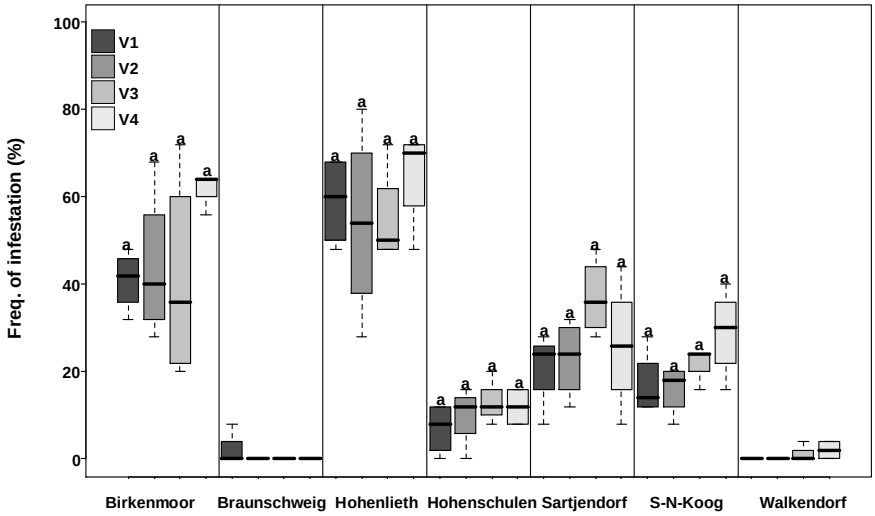


Fig. 49: *Verticillium longisporum* frequency of infestation of field trials in 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. Values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

In 2009/10, infestations were recorded in Birkenmoor, Hohenlieth, Hohenschulen, Sönke-Nissen-Koog and Sartjendorf, with the highest infestation frequency in Birkenmoor and Hohenlieth (Fig. 49). For V3 the highest frequency of infestation with a mean of 37 % was assessed at Sartjendorf. There were no significant control effect caused by the single use of HRO-C48 within this period and for the combined treatment the highest frequency of infestation was assessed at most sites. In Birkenmoor the frequency of infestation in V4 was increased by 51 % compared to the control.

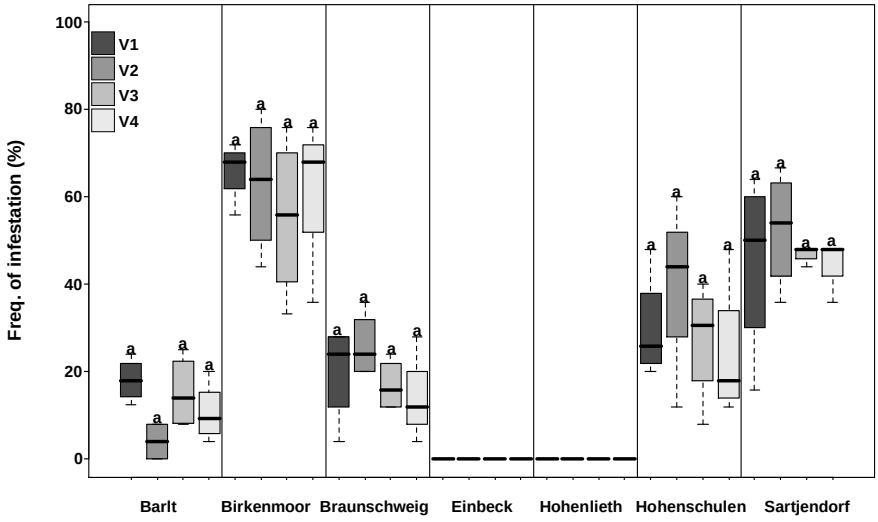


Fig. 50: *Verticillium longisporum* frequency of infestation of field trials in 2010/11. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. Values are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

During the last investigation period the frequency of infestation was highest at Braunschweig, Hohenschulen and Sartjendorf and again, no significant differences were recorded between the treatments (Fig. 50). Differences between the variants act mainly analogous to data of disease score in 2010/11. In V3 at Birkenmoor 16 % less infected plants compared to the control were recorded, while other variants did not differ from each other. Frequency of infestation was increased in the fungicide treatment V2 (2<1: 33 %) in Hohenschulen, whereas the combined treatment V4 realized a reduction of 20 % compared to the control. Both bacterial treated variants could reduce the frequency in Braunschweig (3<1: 15 %; 4<1: 30 %).

The fact that there was no significant interaction between site, year and variant allowed analysing variants pooled over year and site.

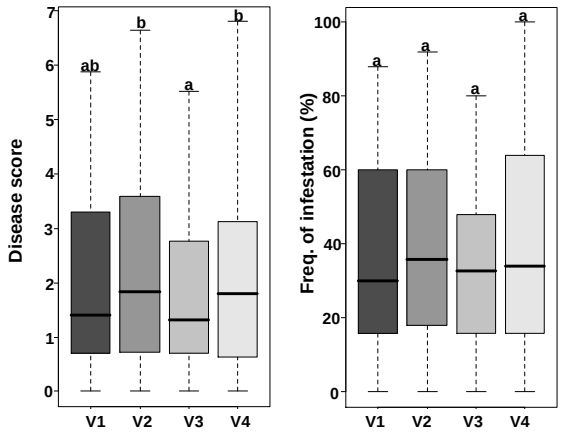


Fig. 51: *Verticillium longisporum* disease score and frequency of infestation of field trials summarized for all sites from 2009-2011. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. Values are presented as box plots. Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

In summary of all sites and years, the disease score for fungicide-treated variants V2 (T=3.3, df=204, p=0.0064) and V4 (T= 2.9, df=204, p=0.018) were significantly higher than in bacteria alone-treated V3 (Fig. 51). On average, the single bacterial treatment reduced the disease score by 8 % compared to the control, although this effect was not significant. In contrast to the disease score, no significant differences were calculated for the frequency of infestation, but ranking of means were analogues to the DS: Most plants were infested in V2 (42,1 %), followed by V4 (41,7 %) and V1 (39,6 %), whereas V3 showed the lowest percentage of infected plants (37,8 %) (Fig. 51). Box plots illustrate that differences between the variants were not significantly different and the differences between the variants were negligible.

3.1.3 ***Plasmodiophora brassicae***

P. brassicae infested plants were found in Walkendorf in season 2008/09 and in Birkenmoor in 2009/10. Statistical analysis of disease index and frequency of infestation data revealed no significant influences of site, year and variant as well as no significant interactions. Therefore data of both sites were summarized and are presented in Fig. 52.

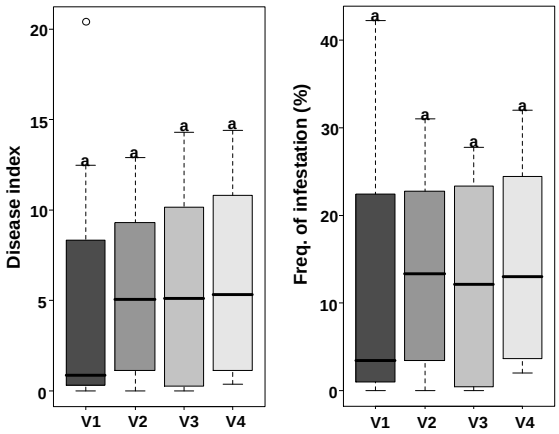


Fig. 52: *Plasmodiophora brassicae* disease index (DI) and frequency of infestation of field trials summarized for Walkendorf 2008/09 and Birkenmoor 2009/10. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. Data are presented as box plots. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

All treatments showed higher disease indexes (DI) than the untreated control, but box plots demonstrate that there were no major differences between variants (Fig. 52). With a mean of 6.2 the combined treatment V4 generated the highest disease index. This is a non-significant increase of 25 % compared to the control, which had a mean of 4.9. In summary, severity of infestation was not high. Means of the infestation frequency ranged between 12 (V1) and 14.5 % (V4). The ranking is equal to the DI, while differences between variants are not significant and low.

Walkendorf

Rep.4	**V3 Harvest**	**V1 Harvest**	**V4 Harvest**	**V2 Harvest**
	V3: 1%	**V1: 2%**	**V4: 5%**	**V2: 5%**
Rep.3	**V2 Harvest**	**V4 Harvest**	**V1 Harvest**	**V3 Harvest**
	V2:31%	**V4:32%**	**V1:35%**	**V3:21%**
Rep.2	**V4 Harvest**	**V3 Harvest**	**V2 Harvest**	**V1 Harvest**
	V4: 2%	**V3: 0%**	**V2: 0%**	**V1: 0%**
Rep.1	**V1 Harvest**	**V2 Harvest**	**V3 Harvest**	**V4 Harvest**
	V1: 0%	**V2: 0%**	**V3: 0%**	**V4: 4%**

Birkenmoor

Rep.4	**V4: 21%**	**V2: 26%**	**V3: 26%**	**V1 Harvest**	**V4 Harvest**	**V2 Harvest**	**V1: 4%**	**V3 Harvest**
Rep.3	**V2 Harvest**	**V4 Harvest**	**V3 Harvest**	**V1: 42%**	**V1 Harvest**	**V4: 21%**	**V3: 21%**	**V2: 19%**
Rep.2	**V3: 28%**	**V3 Harvest**	**V4 Harvest**	**V4: 28%**	**V2: 20%**	**V1: 10%**	**V2 Harvest**	**V1 Harvest**
Rep.1	**V1: 2%**	**V1 Harvest**	**V2: 8%**	**V2 Harvest**	**V3: 3%**	**V3 Harvest**	**V4: 3%**	**V4 Harvest**

Fig. 53: Breadboard of field trials in Walkendorf 2008/09 and Birkenmoor 2009/10. Percentages represent frequency of infestation with *Plasmodiophora brassicae* in plots. V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. No samples were taken from harvest plots.

Since *P. brassicae* is a soil borne disease, the distribution of the spores is usually patchy. Fig. 53 illustrates the spatial arrangement of repetitions (plots) in the field including individual infestation frequencies. In contrast to the treatment, influence of plot position is striking. This is particularly obvious in Walkendorf, where almost every infested plant was found in the third repetition. In Birkenmoor frequency of infestation was quite low in the first repetition, while spores seemed to be concentrated in repetition 3 and 4. Plots of V1 in replicates 3 and 4 were located further to the right of the field and showed the lowest infestation frequency in these replicates compared to other variants.

Fig. 54 shows the results of the field trial carried out by the DLR in Gondershausen. Soil was heavily infested with resting spores of *P. brassicae* leading to an infestation frequency of 100 % in all repetitions and a notably higher disease index level. With a mean of 70, disease index of the *S. plymuthica* variant was slightly and non-significantly increased compared to the untreated control (62).

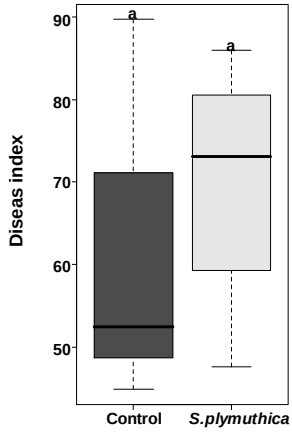


Fig. 54: *Plasmodiophora brassicae* disease index (DI) of DLR field trial in Gondershausen 2009. Untreated seeds (control) were tested against *S.plymuthica* HRO-C48 treated seeds. DI is presented as box plot. Box plots within one frame followed by the same letters are not significantly different according to related multiple contrast tests at significance level α=0.05.

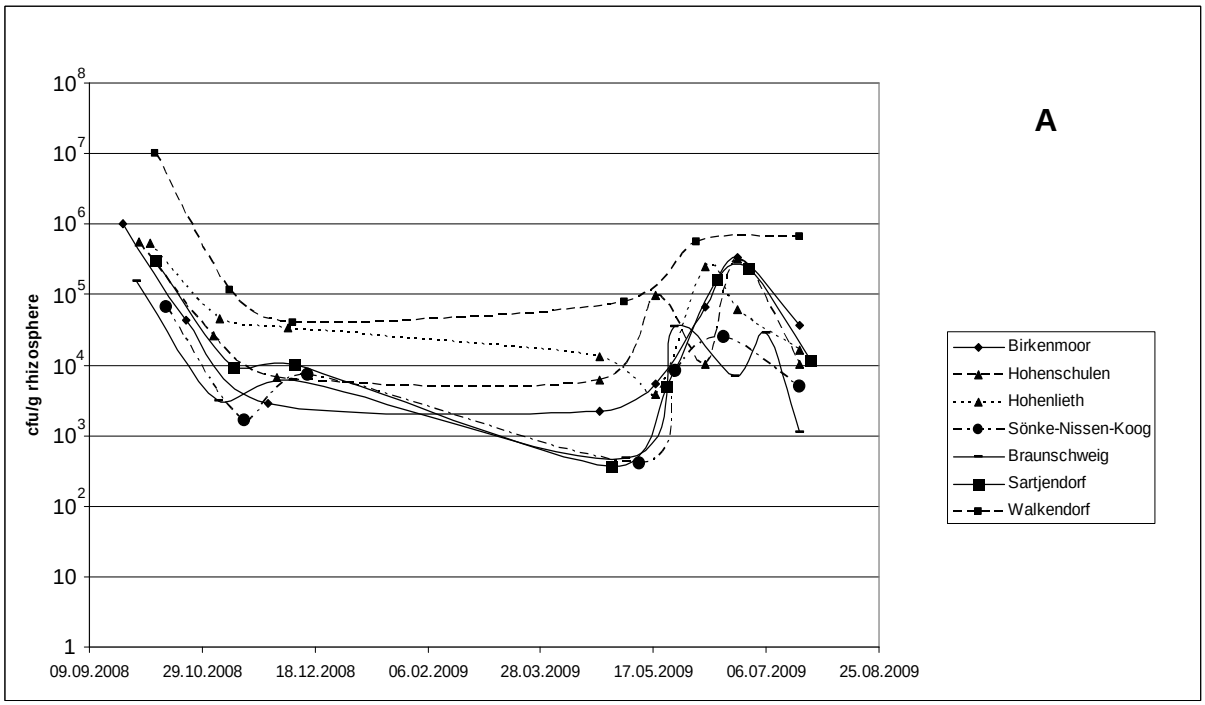
3.1.4 Population dynamics ***Serratia plymuthica*** HRO-C48

S. plymuthica HRO-C48 was able to colonize the rhizosphere of the rapeseed plants efficiently when bacterial enriched seeds were used (Fig. 55). Depending on the year and location, the bacterial densities of $\log_{10}$ 5-7 CFU g^{-1} in early autumn were reached. With a level of $\log_{10}$ 5-6 bacterial densities were comparatively low after sowing in 2008/09 (Fig. 55A). At the beginning of the analysis, Braunschweig and Sönke-Nissen-Koog had the lowest rhizosphere colonization, which decreased below $\log_{10}$ 3 CFU g^{-1} during winter but recovered temporarily in spring/summer. At Hohenlieth and especially at Walkendorf, higher amounts of bacteria were found during the whole season. Bacterial concentrations reisolated in autumn 2009 were slightly higher than in 2008 (except for Sönke-Nissen-Koog) (Fig. 55B). After a decline during winter and spring, rhizosphere colonization at Birkenmoor, Hohenschulen and Hohenlieth nearly reached the same level ($\log_{10}$ 6 CFU g^{-1} rhizosphere) in summer like in October. Generally Walkendorf and Birkenmoor provided the best conditions for bacterial growth in season 2009/2010. The last season of field trials in 2010/11 started with concentrations of $\log_{10}$ 5-7 CFU g^{-1} rhizosphere (Fig. 55C). At Birkenmoor, an increase of bacterial densities in autumn, leading to >$\log_{10}$ 6 cells during winter, was detected. At

Einbeck and Braunschweig higher concentrations of HRO-C48 were assessed in March (log_{10} 6) than in autumn, whereas at Barlt, Hohenschulen and Hohenlieth bacterial levels were around log_{10} 3 CFU g^{-1} rhizosphere at that time. By end of May no bacteria could be detected at Hohenschulen anymore. The population densities at all sites severely varied in spring and summer and the final densities were comparatively low at harvest, except for Hohenlieth and Braunschweig.

The percentage of samples containing HRO-C48 varied in 2008/09 between 47 % (Sönke-Nissen-Koog) and 98 % (Walkendorf). In the following trial period (2009/10), the percentage of HRO-C48 positive samples was lower at most sites. Braunschweig had an average frequency of 23 %, whereas Birkenmoor (89 %) and Walkendorf (97 %) had the highest number of positive samples. During the last season of trials, four sites reached more than 80 % of HRO-C48 positive samples, while Hohenschulen (43 %) and Barlt (51 %) had the lowest frequency of positive samples (data not shown).

In general, the bacterial concentration declined until December and dropped to a minimum level of log_{10} 3-4 CFU g^{-1} rhizosphere. During spring until summer the populations fluctuated whereas an average increase compared to winter was observed in most cases. Samples from Walkendorf continuously had the highest concentrations of HRO-C48. Except for Braunschweig 2009/10 and Hohenschulen 2010/11, HRO-C48 was found during the whole period of investigation at all trial sites.



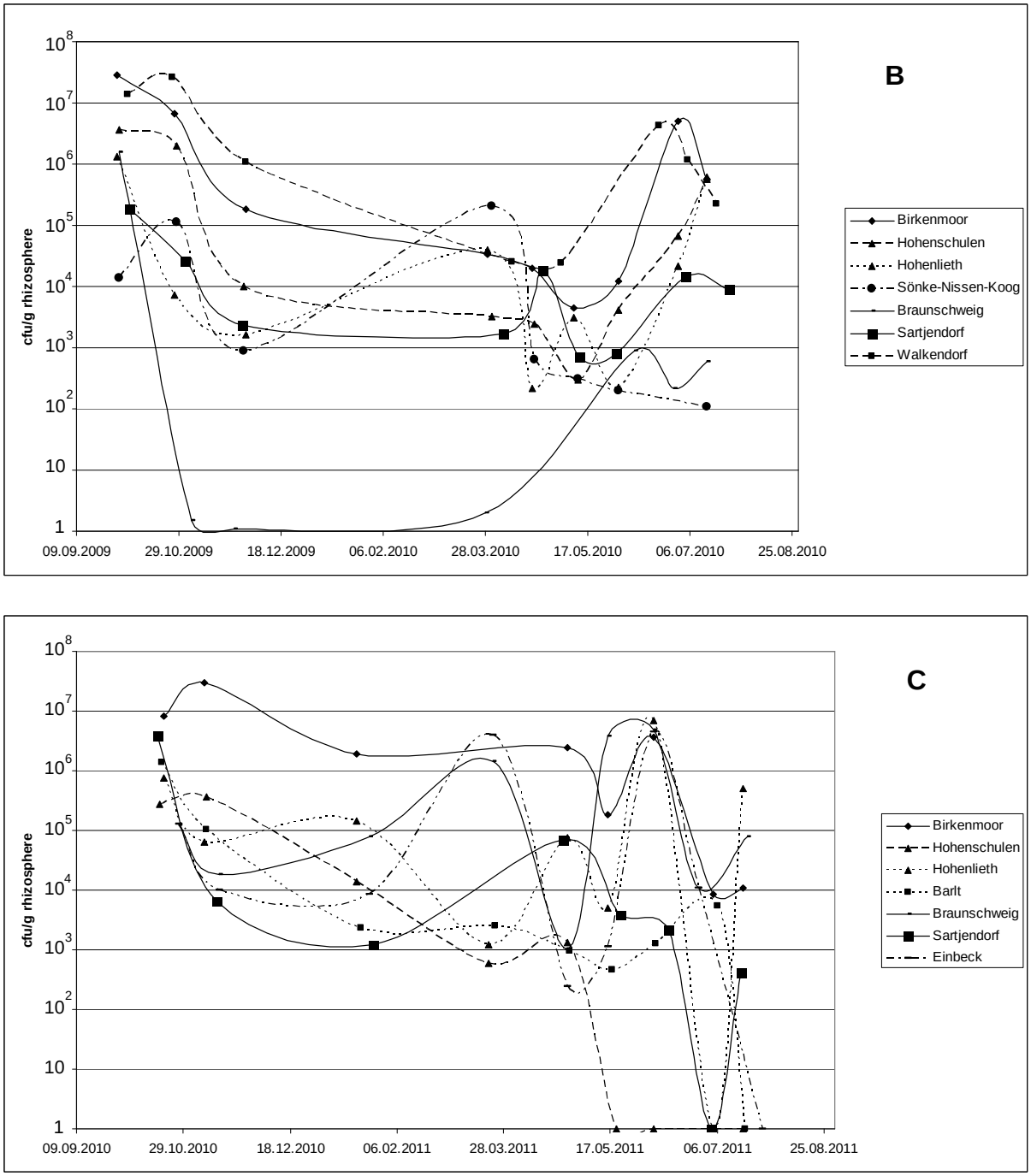


Fig. 55: ***Serratia plymuthica*** **HRO-C48 population dynamics at different trial sites in A) 2008/09 B) 2009/10 C) 2010/11. Markings of trend lines represent means of 8 root samples taken from plants of V3 and V4 expressed as colony forming units (CFU) per gram of rhizosphere in a logarithmic scale.**

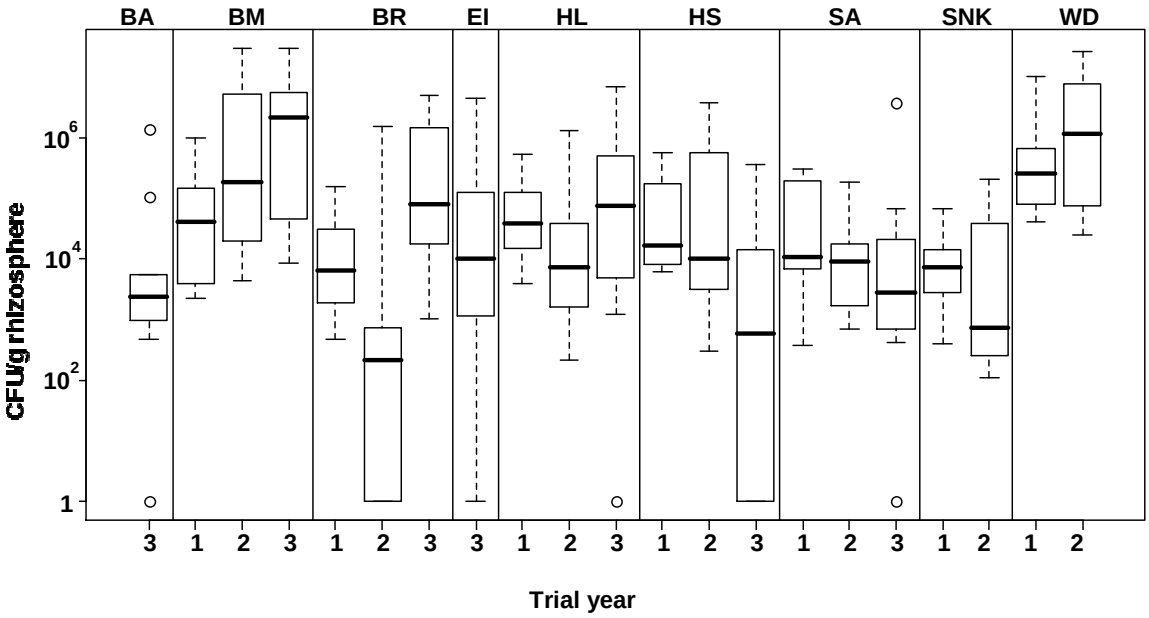


Fig. 56: Mean bacterial densities of *Serratia plymuthica* HRO-C48 at different sites subdivided by trial years shown as boxplots in a logarithmic scale. Trial sites: BA=Barlt, BM=Birkenmoor, BR=Braunschweig, EI=Einbeck, HL=Hohenlieth, HS=Hohenschulen, SA=Sartjendorf, SNK=Sönke-Nissen-Koog, WD=Walkendorf. Trial seasons: 1=2008/09, 2=2009/10, 3=2010/11. CFU=colony forming units.

Fig. 56 additionally clarifies that Walkendorf and Birkenmoor achieved the highest bacterial densities in the rhizosphere. Bacterial level varied between the trial seasons, while tendencies differ depending on the site. Birkenmoor had increasing densities during the investigation period, while the total amount of bacteria decreased at Sartjendorf and Hohenschulen. At Braunschweig and Hohenlieth, the lowest densities were assessed during the second trial period. Except for Braunschweig 2009/10 and Hohenschulen 2010/11, where no bacteria were detected for longer periods, marshland sites Barlt and Sönke-Nissen-Koog generally had the lowest bacterial levels in comparison to the other sites. Statistical analysis with Tukey-Test (α=0.05) of AUDPC transformed bacterial densities revealed no significant differences between the sites.

3.1.5 Yields

Yields recorded at the three trial during the period 2008/09-2010/11 are presented in Table 11. The first season revealed an averaged surplus through all treatments. In relation to the control (V1), V2 reached 1.2 dt, V3 1.0 dt and V4 1.5 dt/ha higher yields in average. At Hohenschulen, the 2009 fungicide treatment alone provided a yield surplus of almost 4 dt ha^{-1}, whereas treatment V3 resulted in increased yields at Birkenmoor, Hohenschulen and

Hohenlieth of up to 3 dt ha^{-1}. The combined treatment of fungicides and HRO-C48 (V4) consistently achieved higher yields compared to the control variant in 2009. Especially at Braunschweig and Walkendorf a surplus of 2.7 dt ha^{-1} was harvested due to the combined treatment.

In 2010, trends recorded during the previous season did not show up again. Yields differed only slightly between the variants in most cases. Yield was reduced in the fungicide treated variants by 8.8 dt (V2) and 6.3 dt ha^{-1} (V4) in Sartjendorf, while there were small increases in yield in Walkendorf in V2 and V4. In 2010, Hohenschulen was the only trial site where all treatments resulted in higher yields, while V3 generated the highest yield with 52 dt ha^{-1}.

Yield levels decreased from 2009 to 2011, while yields recorded during the last season were noticeably lower than those recorded during the first two years. In 2011, higher yields were realized through application of fungicides in Hohenschulen. With 13 dt ha^{-1}, V3 had the lowest yield at this site. Higher yields through fungicides were reached in Barlt as well, but there was just one application (spring). In Einbeck and Sartjendorf the exact opposite was the case and non-fungicide variants achieved up to 4 dt ha^{-1} higher yields compared to fungicide-treated variants. Spraying fungicides in 2011 led to a decrease in yield at 3 out of 6 sites.

The differences between the four variants are rather small, which is illustrated by the statistical evaluation. There were no significant differences between the variants, while the combined influencing variable of site and year (SY) is of significant influence ($F=165.9$, $df=19,239$, $p<2\ \log_{10}\ -16$). Average yields recorded in 2010 and especially in 2011 differed only slightly between the four variants. The ranking in 2011 was contrary to 2009, which means that variant 1 had the highest yield (39.29 dt ha^{-1}) and variant 4 the lowest (38.84) on average.

Table 11: Yields (in dt ha^{-1}) and corresponding standard deviation of trial sites separated by years and variants: V1: control V2: single fungicidal treatment V3: single bacterial treatment V4: combined fungicidal and bacterial treatment. According to related multiple contrast tests (α = 0.05) no significant differences were found.

Site	Harvest 2009 (dt ha^{-1})				Harvest 2010 (dt ha^{-1})				Harvest 2011(dt ha^{-1})			
	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
Birkenmoor	65,83 ± 1,03	67,73 ± 1,53	67,95 ± 1,66	66,52 ± 2,84	49,49 ± 5,05	50,90 ± 5,43	49,41 ± 5,27	50,85 ± 4,46	47,67 ± 2,87	46,78 3,54	47,76 ± 2,78	47,03 ± 2,66
Hohenschulen	49,27 ± 1,71	53,24 ± 4,27	52,92 ± 4,70	50,30 ± 6,00	48,73 ± 2,26	51,56 ± 1,90	51,98 ± 5,80	50,36 ± 2,36	15,06 ± 5,89	17,14 ± 8,48	13,00 ± 3,95	16,33 ± 13,57
Hohenlieth	62,21 ± 2,60	63,79 ± 0,44	64,49 ± 4,21	64,79 ± 1,00	62,29 ± 2,56	62,36 ± 0,91	61,37 ± 2,46	61,31 ± 1,23	-	-	-	-
Sönke-Nissen -Koog	60,95 ± 3,77	59,55 ± 5,09	58,80 ± 5,31	60,38 ± 5,10	62,63 ± 4,29	63,20 ± 2,76	62,53 ± 1,72	62,08 ± 3,73	-	-	-	-
Barlt	-	-	-	-	-	-	-	-	57,70 ± 5,92	59,28 ± 1,34	55,98 ± 3,65	58,98 ± 4,51
Braunschweig	50,97 ± 3,70	49,81 ± 7,03	51,07 ± 5,08	53,76 ± 3,20	55,01 ± 1,39	56,73 ± 3,40	56,90 ± 4,23	56,30 ± 2,32	25,97 ± 2,24	25,92 ± 1,44	26,20 ± 1,03	26,27 ± 2,73
Sartjendorf	57,29 ± 2,21	58,12 ± 1,45	58,14 ± 3,02	57,81 ± 2,56	53,25 ± 3,35	44,48 ± 3,47	51,27 ± 6,43	47,00 ± 4,36	42,29 ± 1,36	40,70 ± 1,81	44,17 ± 2,50	39,20 ± 1,72
Walkendorf	44,62 ± 0,57	46,97 ± 1,31	44,38 ± 1,07	47,89 ± 1,25	51,23 ± 5,05	52,73 ± 1,85	48,02 ± 4,34	53,53 ± 2,75	-	-	-	-
Einbeck	-	-	-	-	-	-	-	-	47,05 ± 1,99	44,68 ± 2,02	46,65 ± 1,75	45,23 ± 1,12

3.2 Glasshouse trials

3.2.1 *Verticillium longisporum*

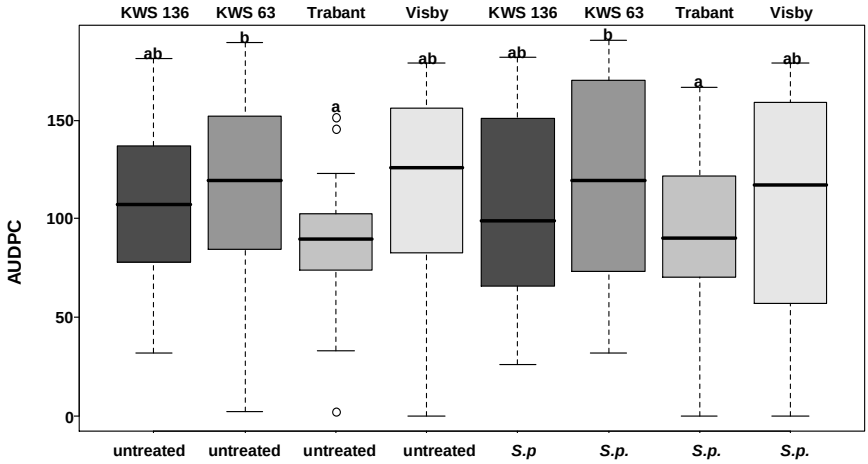


Fig. 57: Area under disease progress curve (AUDPC) of *Verticillium longisporum* presented as box plots recorded during glasshouse trials. Variants: KWS136 untreated + *Serratia plymuthica*; KWS63 untreated + *S. plymuthica*; Trabant untreated + *S. plymuthica*; Visby untreated + *S. plymuthica.* Box plots followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

There were no significant differences between the treatments (control; *S. plymuthica*) and no significant interaction, while the cultivar had a significant influence (F=2.9, df=3, 216, p=0.036). The cultivar KWS63 showed the highest AUDPC with means of 118 (*S. plymuthica* variant) and 114 (control), followed by Visby and KWS136. AUDPC of KWS63 was significantly increased by 22 % compared to Trabant (T=2.8, df=216, p=0.027). Except for Visby, treatment with *S. plymuthica* led to slight, but non-significant increases of DI in all cultivars (Fig. 57).

Bacterial densities were observed before planting, two weeks later and at the end of the trials. After treatment with the bacterial suspension seeds were enriched with about $\log_{10}$ 6 CFU seed^{-1}. Rhizosphere densities of two week old seedlings differed between $\log_{10}$ 6-7 CFU g^{-1} rhizosphere, while concentrations slightly decreased to $\log_{10}$ 5-7 at the end of the trial.

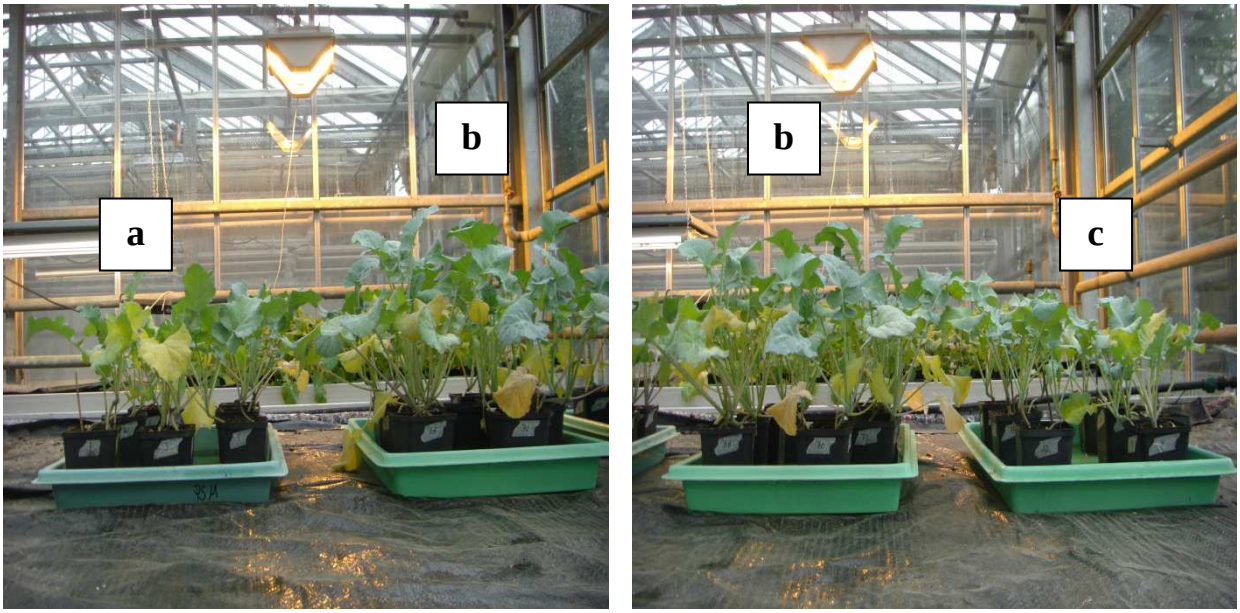


Fig. 58: Stunting of rapeseed plants caused by *Verticillium longisporum*: pictures shows untreated Visby plants (a) and *S. plymuthica* treated plants in infested soil (c) compared to the healthy control (b).

3.2.1 *Plasmodiophora brassicae*

The effect of the bacterial treatment with *S. plymuthica* was also assessed in glasshouse experiments with the susceptible cultivar Visby and the resistant cultivar Mendel. Statistical analysis revealed a highly significant interaction between cultivar and *P. brassicae* spore concentration ($F=136.8$, $df=1, 778$, $p< 2.2 \log_{10} -16$). Due to this interaction, multiple contrast tests of cultivars were conducted separated by spore concentration. Both analysis revealed that increased disease scores recorded for Visby were highly significant (spore level $\log_{10} 5$: $T=10.6$, $df=216$, $p<1 \log_{10} -10$; spore level $\log_{10} 7$: $T=30.6$, $df=216$, $p<1 \log_{10} -10$) compared to Mendel, while interaction of cultivar and spore concentration is caused by higher differences between means at concentration level $\log_{10} 7$ spores ml^{-1} (Fig. 59). There was no significant difference between the treatments (control; *S. plymuthica*). In average *S. plymuthica* treated variants did not differ from untreated variants.

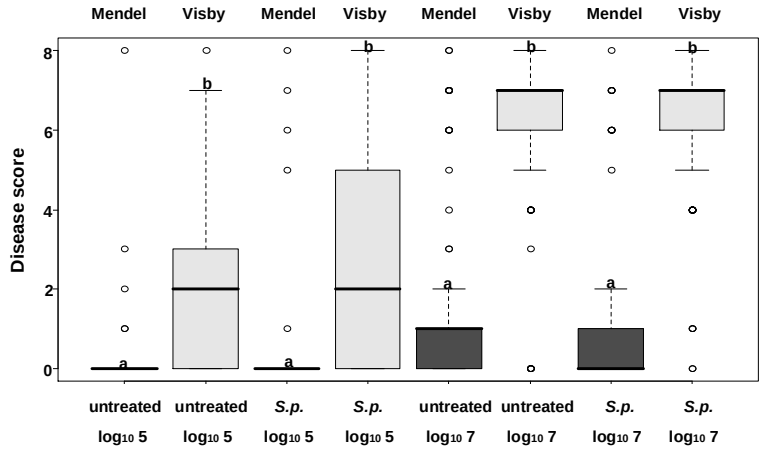


Fig. 59: Disease scores (DS) for *Plasmodiophora brassicae* infection recorded in glasshouse trials presented as box plots. Variants: Mendel untreated + *S. plymuthica*; Visby untreated + *S. plymuthica* tested at two levels of *P. brassicae* spore concentration (log_{10} 5 ml^{-1}; log_{10} 7 ml^{-1}). Box plots within one frame followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Fig. 59 shows that disease scores of the cultivar Visby were clearly increased at the same level of spore concentration. At the lower spore concentration level (log_{10} 5 ml^{-1}) only few plants of Mendel- no matter whether treated with *S. plymuthica* or not- were infested, while Visby variants achieved an average DS of 2.4. This relation is also reflected in the DS at the higher spore concentration level.

Bacterial densities were determined at the end of the trials. Concentrations of samples varied between log_{10} 4- log_{10} 7 CFU g^{-1} rhizosphere, while average concentration was > log_{10} 6.

In Fig. 60 results of a *P. brassicae* glasshouse trial carried out by the Dienstleistungszentrum Ländlicher Raum (DLR) Rheinland-Pfalz are presented. Performance of untreated and *S. plymuthica* treated Visby seeds were tested at different spore concentrations. According to ANOVA, the effect of the *P. brassicae* spore concentration was significant (F=71.8, df=4, 178, p<2 log_{10}-16), while treatments had no significant influence. Therefore, multiple contrast tests were conducted separately for each level of spore concentration.

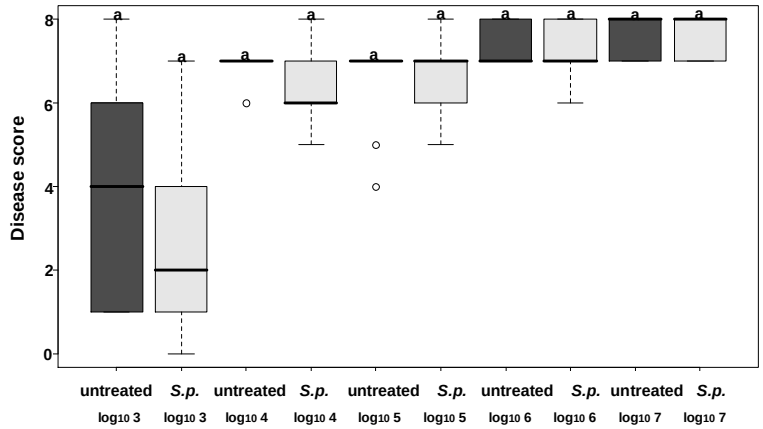


Fig. 60: *Plasmodiophora brassicae* disease score (DS) recorded during glasshouse trials at the Dienstleistungszentrum Ländlicher Raum Rheinland-Pfalz presented as box plots. Untreated and *S. plymuthica* treated cultivar Visby was tested at different levels of *Plasmodiophora brassicae* spore concentration (log_{10} 3 ml^{-1} - log_{10} 7 ml^{-1}). Box plots followed by the same letter are not significantly different according to related multiple contrast tests at significance level α=0.05.

Disease score for *S. plymuthica*-treated plants was reduced by 28 % in comparison to the untreated control at log_{10} 3 spores ml^{-1} (Fig. 60). This reduction at the lowest spore concentration level was almost significant (T=-2.4, df=216, p=0.0784), whereas differences between the treatments were negligible at higher fungal concentration levels. With disease scores surpassing 7 at ≥log_{10} 6 spores ml^{-1} level of disease severity was higher compared to own glasshouse trials (Fig. 59).

3.2.1 *Sclerotinia sclerotiorum*

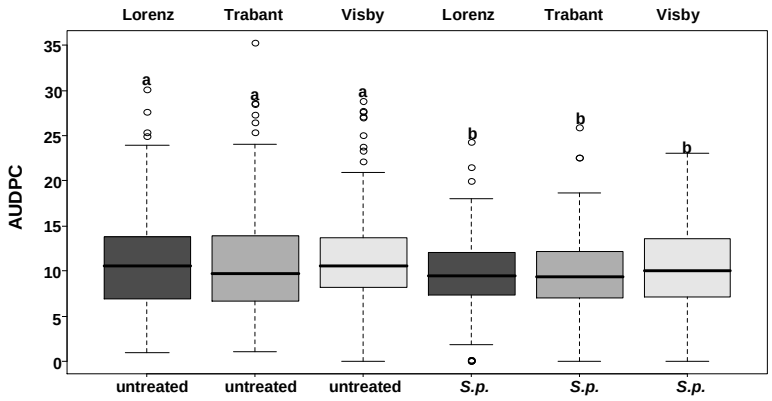


Fig. 61: Area under disease progress curve (AUDPC) for *Sclerotinia sclerotiorum* infection recorded during glasshouse trials presented as box plots. Untreated and *Serratia plymuthica* treated cultivars Lorenz, Trabant and Visby were tested. Box plots within followed by different letters are significantly different according to related multiple contrast tests at significance level α=0.05.

Statistical analysis revealed a significant effect of the bacterial treatment (F=5.9, df=1, 262, p=0.016), while the influence of the cultivar was not significant and no interaction between cultivar and treatment was recorded. This enabled summarized multiple contrast tests for the two different treatments which revealed significantly lower AUDPC of *S. plymuthica* treated plants (T= -2.4, df=262, p=0.016). In average, the AUDPC was reduced by 0.96 throughout the treatment with the bacterium. In Fig. 61 results of the *S. sclerotiorum* trial are presented. Visby had a slightly higher AUDPC (10.7) compared to Lorenz (10.1) and Trabant (10.1).

HRO-C48 rhizosphere densities of two week old seedlings differed between $\log_{10} 6$ - $\log_{10} 7$ CFU g^{-1} rhizosphere, while concentrations remained stable until the end of the trials.

3.3 Improvement of seed treatment

3.3.1 Spray application

The basic idea of this trial was to examine the possibility of a sustainable enrichment of *S. plymuthica* inside rapeseed seeds by use of an airbrush gun. The trial setup included the three different initial variables: moisture of seeds, bacterial concentration and ratio between applied suspension and seed weight to test their influence. To verify the survival capability of the bacteria, treated seeds were stored in different ways.

ANOVA of the whole log-transformed dataset revealed a significant interaction between the factors: ratio, bacterial concentration and storage conditions ($F=1.8$, $df=12, 288$, $p=0.048$). Consequently, the initial moisture of seeds, which, in general, had a significant influence on the bacterial concentration of seeds ($F=9.0$, $df=2, 144$, $p=0.0002$), was the only variable that did not interact with the other factors.

Due to the wide range of interactions between the initial treatments and storage conditions, general effects of the other factors -besides the water content of the seeds- cannot be evaluated for the whole data set. Consequently, significant effects of the temperature, for example, can only be determined under conditions when no interactions with the other trail variants exist.

The influence of the initial seed treatment conditions on the initial amount of bacteria absorbed by the seeds were analysed by using the dataset after two days of storage. At that time the water content of seeds was above 25 % and storage conditions had the lowest influence on the amount of bacteria re-isolated from the seeds. For the ANOVA analysis and post hoc Tukey HSD tests the data are separated for the different storage conditions: closed at 4°C (V1), opened at 4°C (V2), closed at 25°C (V3) and opened at 25°C (V4). This was done in order to avoid interactions between the initial seed treatment conditions and the storage conditions in the statistical analysis. According to Tukey HSD Test ($\alpha=0.05$) significant differences in bacterial densities were found in V1, V3 and V4 and are presented in Fig. 62-64.

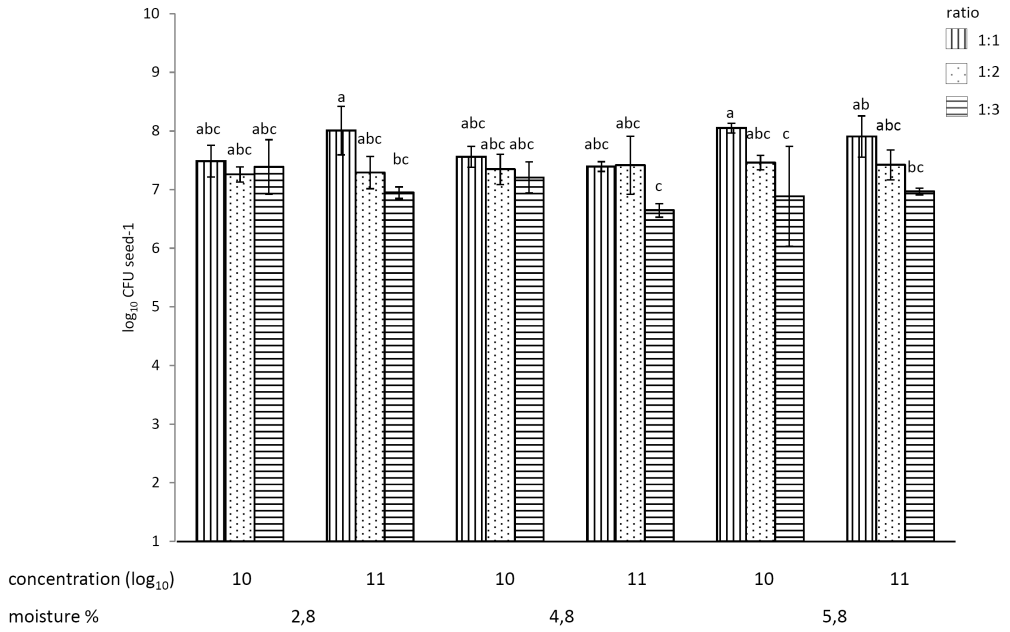


Fig. 62: Influence of the initial bacterial concentration and seed moisture and the ratio of bacteria to seeds (ml g^{-1}) on the bacterial density in one seed after 2 days storage at 4°C in a closed container. Data are given as log_{10} CFU (colony forming units) per seed. Bars with different letters are significantly different according to Tukey HSD at α=0.05 (3 replicates per treatment).

In a closed container at 4°C only the ratio between seeds and bacterial suspension had a significant influence on the bacterial content in the seeds. However, this counts only for specific conditions (see bacterial density ≥log_{10} 11 CFU ml^{-1} at 2.8 % water content and ≥log_{10} 10 CFU ml^{-1}at 5.8 % (Fig. 62). The bacterial density and the water content of the seeds had no significant influence. Bacterial level of V1 differed between 5 x log_{10} 6 and log_{10} 8 CFU $seed^{-1}$.

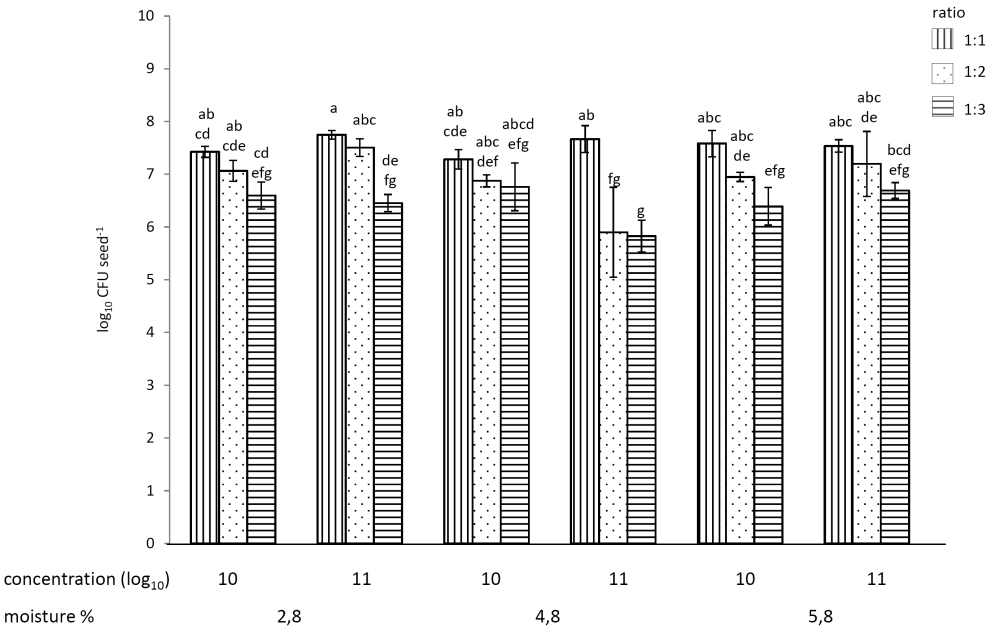


Fig. 63: Influence of the initial bacterial concentration and seed moisture and the ratio of bacteria to seeds (ml g^{-1}) on the bacterial density in one seed after 2 days storage at 25°C in a closed container. Data are given as log_{10} CFU (colony forming units) per seed. Bars with different letters are significantly different according to Tukey HSD at α=0.05 (3 replicates per treatment).

In a closed container at 25°C the bacterial level was lower compared to V1 (Fig. 63) two days after treatment, while the ratio had a significant influence at moisture levels of 2.8 and 4.8 % when seeds were treated with ≥log_{10} 11 CFU ml^{-1}, as well as at a moisture level of 5.8 %, when treated with ≥log_{10} 10 CFU ml^{-1}. Furthermore, a significant influence of the moisture of seeds was found at an initial concentration of ≥log_{10} 11 CFU ml^{-1} and at a ratio of 1:2, indicating significant higher bacterial densities at moisture levels of 2.8 % and 5.8 % compared to 4.8 %.

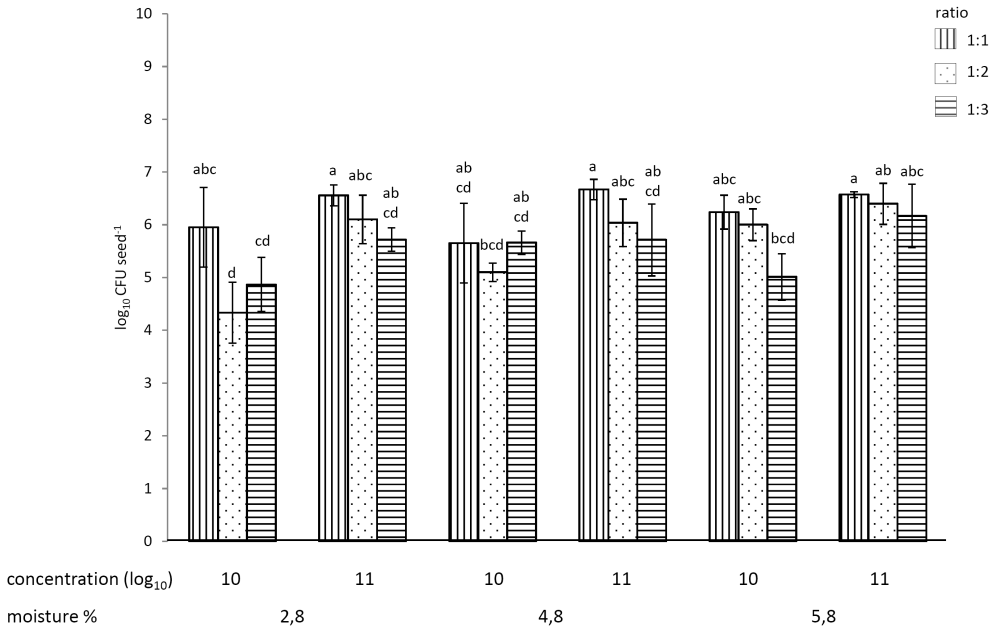


Fig. 64: Influence of the initial bacterial concentration and seed moisture and the ratio of bacteria to seeds (ml g^{-1}) on the bacterial density in one seed after 2 days storage at 25°C in an opened container. Data are given as log_{10} CFU (colony forming units) per seed. Bars with different letters are significantly different according to Tukey HSD at α=0.05 (3 replicates per treatment).

In an opened container at 25°C the ratio and the moisture level had a significant influence on the bacterial content of the seeds. The bacterial level is comparably low differing between log_{10} 4-7 CFU $seed^{-1}$. While with the ratio 1:1 the highest amounts of CFU were obtained, the difference was significant only at moisture content of 2.8 % (concentration of ≥log_{10} 11 CFU ml^{-1}) compared to the ratio 1:3. At this moisture level of seeds there was also a significant difference between the different initial bacterial concentrations at the ratio 1:2 (Fig. 64). In contrast to V1 and V3, an initial concentration of ≥log_{10} 11 CFU ml^{-1} led to higher bacterial densities inside the seeds.

To estimate the influence of the different storage types and temperatures, bacterial densities after 30 days of storage were assessed. For statistical analysis the dataset was subdivided by the different levels of moisture. In contrast to the analysis of the initial variables, Tukey HSD Test (α=0.05) was carried out separated for each bacterial concentration and ratio (Fig. 65-67).

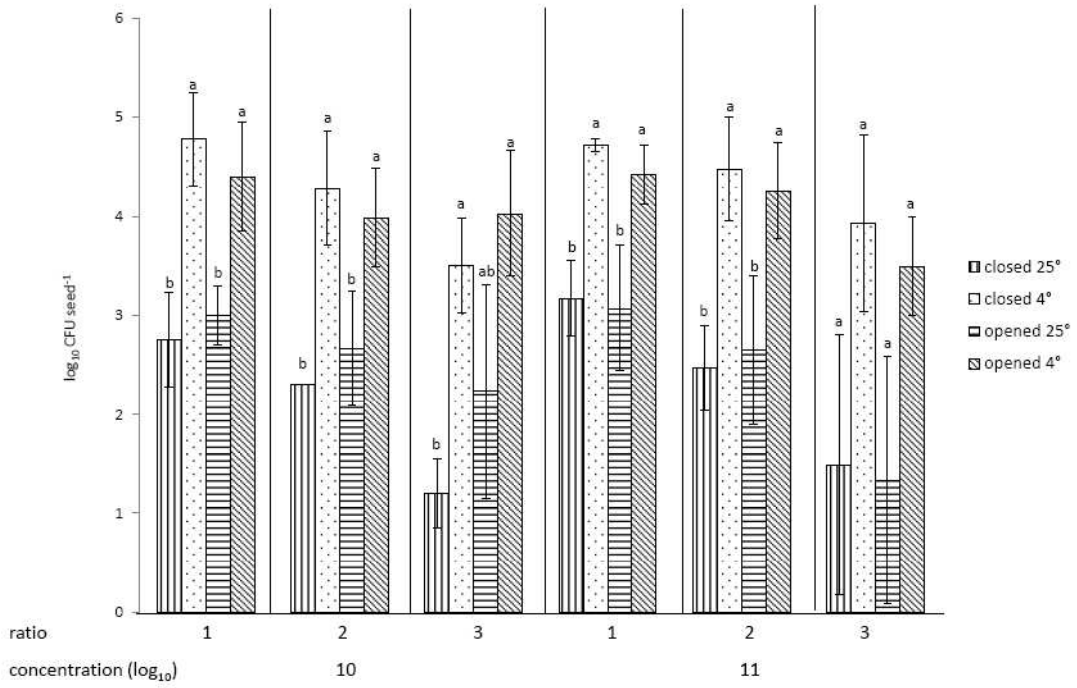


Fig. 65: Influence of 30 days storage at 4° and 25°C in closed or opened containers on the bacterial density in the seed with seed water content of 2.8 %. Data are given as log_{10} CFU (colony forming units) per seed. Bars followed by different letters are significantly different within the column according to Tukey HSD at α=0.05 (3 replicates per treatment).

The bacterial level in seeds containing 2.8 % moisture at the time of treatment ranged between log_{10} 1 - log_{10} 5 after 30 days of storage (Fig. 65). The effect of the storage temperature- regardless of the storage type- is striking. Compared to 25°C, significantly higher cell densities were recorded at 4°C in 5 out of 6 cases. Despite for ratio 1:3 treated with a bacterial concentration of $\geq log_{10}$ 10 CFU ml^{-1}, closed storage showed increased bacterial densities, but differences to storage in opened containers were non-significant.

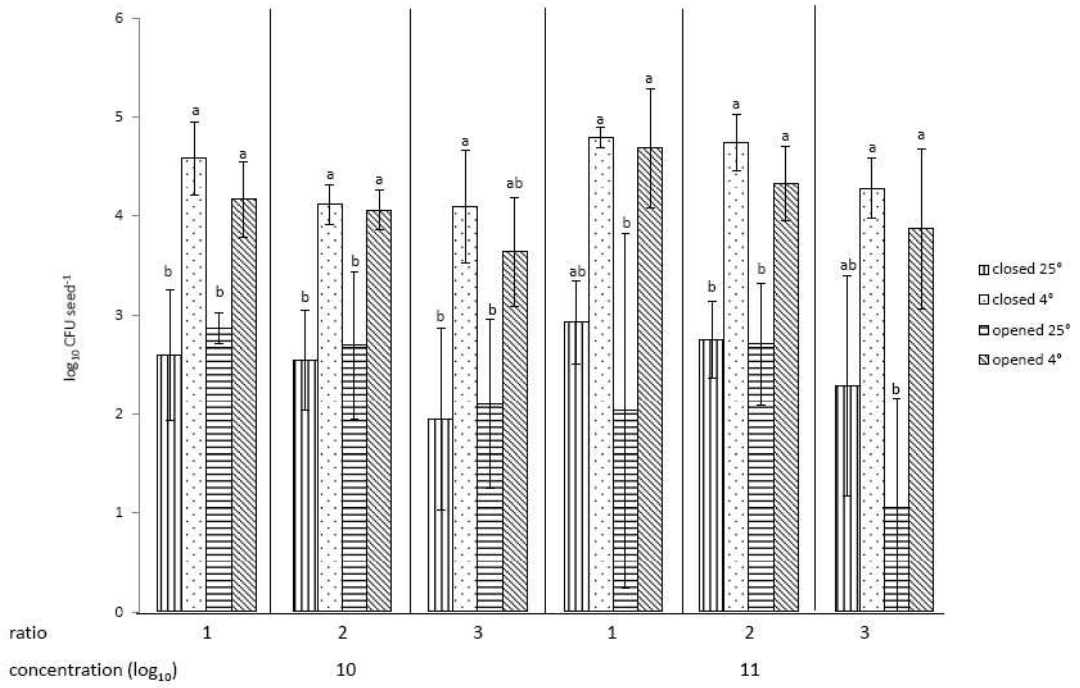


Fig. 66: Influence of 30 days storage at 4° and 25°C in closed or opened containers on the bacterial density in the seed with seed water content of 4.8 %. Data are given as log_{10} CFU (colony forming units) per seed. Bars followed by different letters are significantly different within the column according to Tukey HSD at α=0.05 (3 replicates per treatment).

Cell densities of seeds containing 4.8 % water at the time of treatment are illustrated in Fig. 66. Again, bacterial densities of cold stored samples contained more bacteria. This effect was significant- regardless of the storage type- in 3 out of 6 cases. At a concentration of $\geq log_{10}$ 11 CFU ml^{-1} the effect of the storage temperature was not significant at ratios of 1:1 and 1:3 when stored closed, while differences within opened storage were non-significant at ratio 1:3 when applied suspension was $\geq log_{10}$ 10 CFU ml^{-1}.

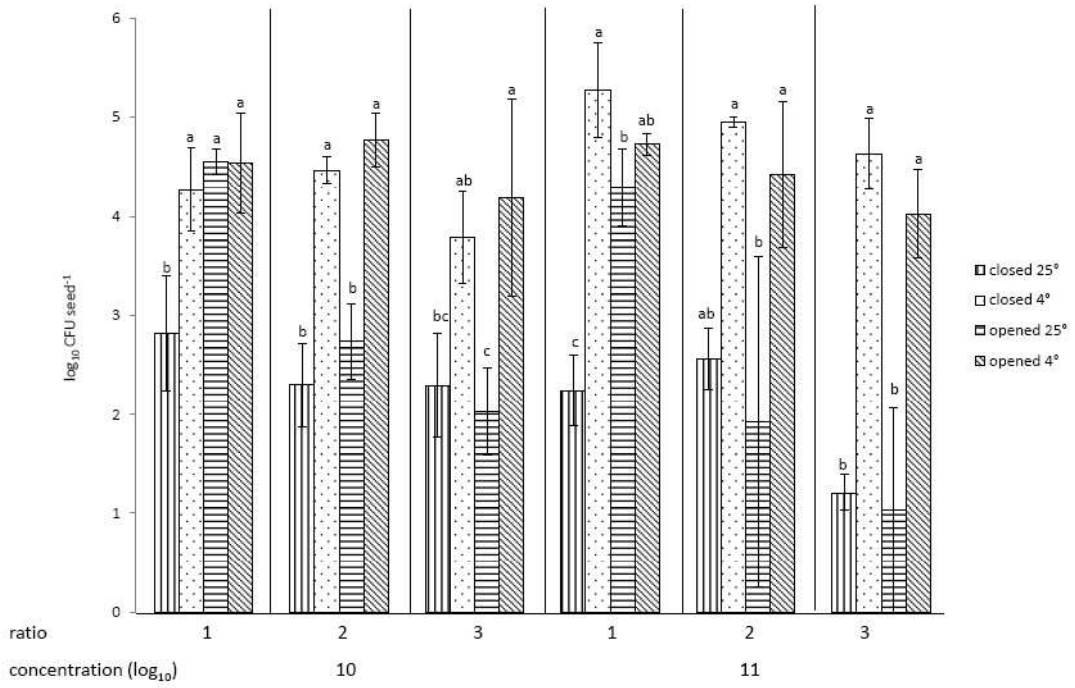


Fig. 67: Influence of 30 days storage at 4° and 25°C in closed or opened containers on the bacterial density in the seed with seed water content of 5.8 %. Data are given as log_{10} CFU (colony forming units) per seed. Bars followed by different letters are significantly different within the column according to Tukey HSD at α=0.05 (3 replicates per treatment).

The bacterial level of seeds containing 5.8 % moisture at the time of treatment was higher compared to 2.8 and 4.8 % after 30 days of storage (Fig. 67). In contrast to previous analysis, a significant difference according to the storage type was found at $\geq log_{10}$ 11 CFU ml^{-1} and the ratio 1:1 at 25°C. Cold stored variants had higher bacterial densities again, while most differences were significant in comparison to storage at 25°C. An exception was found at $\geq log_{10}$ 10 CFU ml^{-1} at the ratio 1:1. Opened and warm stored (V4) samples had higher CFU than opened and cold (V2) as well as closed and cold (V1).

For use of the airbrush procedure, concentration of $>log_{10}$ 4 CFU $seed^{-1}$ can be ensured by use of the ratio of 1:1 and 1:2 (ml bacterial suspension/g seed) and a storage temperature of 4°C, even after 30 days of storage. The decline in cell densities due to the higher storage temperature is clearly visible and significant in most cases, while no major differences regarding the storage type were recorded. An effect of storage temperature was recorded already after two days storage

3.3.2 Clay mineral trial

Three different clay minerals were enriched with *S.plymuthica*, dried and stored at different temperatures. Bacterial density was assessed after 2, 7 and 30 days of storage.

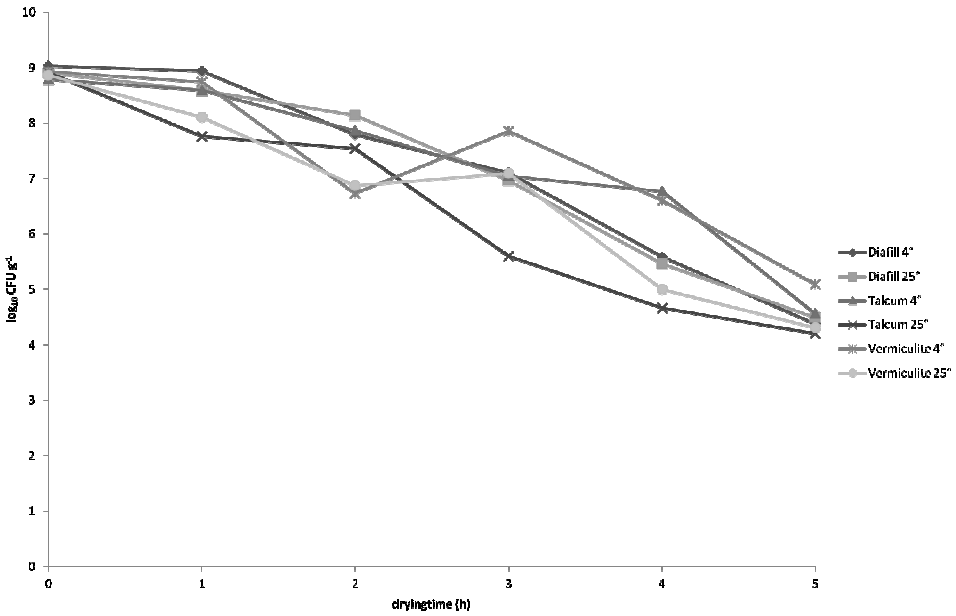


Fig. 68: Influence of drying at 40°C on the bacterial densities given as log10 CFU (colony forming units) per gram of talcum, vermiculite and diafill after storage for two days at 4 or 28°C.

In Fig. 68, CFU isolated from clay minerals two days after bacterial inoculation are presented. The initial bacterial density before drying the clays was at approximately $\log_{10}$ 9 CFU g^{-1}. Drying decreased the cell density, resulting in $\log_{10}$ 4.2 to $\log_{10}$ 5.1 after 5 hours. Except for Vermiculite, the bacterial density declined with the duration of drying.

For the statistical analysis, data on bacterial density were area under curve (AUC) transformed. ANOVA of the whole data set revealed a significant effect of the storage duration ($F=213.3$, $df=2, 36$, $p<0.001$) and a significant interaction between clay mineral and storage temperature ($F=3.8$, $df=2, 36$, $p=0.031$). Due to this interaction, Tukey HSD-Tests ($\alpha=0.05$) were conducted separated for each clay mineral (Table 12).

Table 12: AUC (area under curve) and standard deviation of the re-dried (0-5h) clay minerals talcum, vermiculite and diafill, separated by storage time (2, 7 and 30 days) and storage temperature (4° and 25°C). Mean AUC values of the same clay mineral followed by different letters are significantly different according to Tukey HSD test (α=0.05).

Storage Temperature (°C)	Storage duration (days)	Talcum (AUC)	Vermiculite (AUC)	Diafill (AUC)
4	**2**	**36,9a** ±4,3	**36,9a** ±1,6	**36,1a** ±2,8
25	**2**	**32,1ab** ±0,8	**33,6ab** ±2,8	**35,9a** ±2,8
4	**7**	**35,6a** ±1,1	**34,0ab** ±2,9	**33,5a** ±3,0
25	**7**	**27,7bc** ±2,9	**30,0b** ±3,4	**32,2a** ±1,2
4	**30**	**23,3c** ±0,09	**22,4c** ±1,7	**23,7b** ±1,2
25	**30**	**15,5d** ±1,3	**16,7c** ±2,2	**17,7b** ±2,1

Table 12 shows that elongated storage (30 days) led to significant lower cell densities compared to the initial concentration no matter which clay mineral was tested. Further, storage temperatures of 25°C resulted in a decrease of bacterial densities. Differences regarding to the storage temperature were most pronounced after 30 days of storage. In comparison to talcum samples stored at 25°C, cold stored talcum exhibited significantly more CFU/seed after 30 days. Tukey HSD-Tests (α=0.05) of the different clay minerals within the two storage temperatures separated by storage duration resulted in very slight, non-significant differences.

Freshly prepared and two hours dried bacterial enriched clay minerals were applied in the chemical priming process as described in chapter 2.3.2.1. No bacteria could be found two days after treatment.

4. Discussion

4.1 Field trials

During the first season of trials, *Serratia plymuthica* treatments resulted in considerable reductions of leaf infection caused by *P. lingam*. These results match with data of previous greenhouse experiments using artificial infection with *P. lingam* pycnidiospores, demonstrating disease reductions of more than 70 % by the bacterial seed treatment alone (Abuamsha et al. 2010a). In 2008/09, reduction through the single fungicidal treatment was never significant at any of the trial sites, whereas the single bacterial treatment and the combination of both resulted in significant differences to the control at three out of seven sites. However, despite high efficiencies of bacterial treatments against *P. lingam* leaf infestation during the first year, results were not consistent during the following two years. In 2009/10 and in 2010/11, no significant effects were recorded on the leaf infestation by the single bacterial treatment (V3) at any of the trial sites, while fungicide treated variants (V2) and combinations with bacteria (V4) generated a significant reduction only twice out of 7 sites. Comparing the leaf infestation level between the first and the consecutive years, the levels were extremely low in the last two seasons at all trial sites. The lower the infestation levels, the lower are the measurable effects as well, causing problems for the evaluation of differences between the treatments.

In contrast to many other authors (Gladders and Musa 1980, Hammond and Lewis 1986, Sun et al. 2000, West et al. 2002a), the presented investigation could not confirm a correlation between the intensity of leaf infestation and the occurrence of crown canker**.** Regression analyses of the data revealed only weak, non-significant relations. Despite high levels of *P. lingam* leaf infestation, occurrence of crown canker was lower in 2008/09 compared to 2010/11, while leaf infestation and crown canker in 2009/10 were of no importance. A dry spring in 2009 with no rainfall in April led to a stagnancy of plant growth and maybe the mycelium growth too, what might explain the minor occurrence of crown and stem symptoms. *P. lingam* grows systemic and asymptomatic down the petiole into the stem of rapeseed plants (Hammond and Lewis 1985). In 2010/11 black frost caused huge losses of leaves resulting in smaller amounts of leaf pycnidia per plant. Here mycelium of *P. lingam* might have grown into stems very early in autumn 2010, making the loss of leaves in winter less important. Damage caused by insects and frost might also have provided further entrance wounds for pycnidiospores in spring 2011 (Broschewitz et al. 1993). According to Hammond

and Lewis (1986) plant populations in which the autumn phase of the epidemic had reached significant levels showed higher levels of stem infection than those which had developed a negligible autumn epidemic. In 2008 and 2010, primary leaf infection by ascospores was already severe in early autumn, with higher counts of pycnidia than recorded during the first year.

Crown canker causes economic losses more than other *P. lingam* symptoms (Fitt et al. 2006). Due to the generally very low infestation level in 2009/10, results from this trial year are not discussed in more detail. Regarding crown canker and stem lesions, results were more inconsistent than those recorded for leaf infestation. While the single fungicide treatment could reduce crown canker disease score and infestation frequency in 6 out of 7 cases in the first season of trials (2008/09), single fungicide treatment led to increased disease scores in 6 out of 7 cases and increased infestation frequency at 4 out of 7 sites in 2010/11. None of the reductions recorded for the single fungicidal treatment regarding crown canker parameters (disease score, pycnidia, frequency of infestation) were significant. In contrast the single bacterial treatment reduced the crown canker disease score significant in 1 out of 7 cases, while root collar pycnidia and infestation frequency was significantly reduced at 2 out of 7 sites. All significant differences occurred in the first season of trials. Though the effect of the bacteria were lower in the last season of trials, reductions of the disease score and frequency of infestation were provided at 4 out of 7 sites. The combined treatment resulted in crown canker reductions at every site of all evaluated parameters belonging in the first season of trials. The disease score and the infestation frequency were significantly reduced in 2 out of 7 cases each, while there was one significant reduction of the number of pycnidia. Similar to the single bacterial treatment, all significant effects were recorded in 2008/09, while the effect on more severe crown canker, recorded during the season 2010/11, was less expressed.

A possible explanation for the low effect on crown cancer in 2010/11 might be the low level of bacteria recorded after the winter months. Despite the good establishment of HRO-C48 in autumn, bacterial levels dropped in winter/spring beneath $\log_{10}$ 4 CFU g^{-1} rhizosphere and did not recover again at Hohenlieth, Barlt, Sartjendorf and Hohenschulen, as recorded in 2008/09. Thus the low abundance of bacteria in the rhizosphere might have been the reason for the failure of the treatment with HRO-C48 against crown canker in 2011, as these symptoms are developing during spring and summer.

P. lingam stem infestation was not severe during the complete investigation period. Neither Azoles nor *S. plymuthica* effected the development of these disease symptoms considerably.

Summarized results for *V. longisporum* including all sites and trial years revealed a slight difference within the non fungicide treated variants (V1, V3), while fungicide treated variants (V2, V4) had an increased infestation compared to the control and a significant higher infestation compared to the single bacterial treatment. The individual analyses of the sites separated by trial year revealed no significant differences between the variants except for Hohenlieth in 2008/09. Consequently it can be assumed that the significant difference found in the summarized analysis, which included all trial years and sites, strongly depends on this highly significant difference ($p<0.001$) found in Hohenlieth. In the first season of field trials, the single bacterial treatment reduced disease severity compared to the untreated at 3 out of 7 sites, but differences within the non-fungicide treated variants (V1,3) were generally low in the following trial seasons. Since the variant treated with *S. plymuthica* and azoles together showed higher disease scores of *V. longisporum* compared to the single bacterial treatment, it might be considered that the negative effect caused by azole treatments overruled any antagonistic effect caused by the bacterial treatment.

A possible explanation for more severe infestation with *V. longisporum* in fungicide treated plots could be the stunting effect that is caused by azoles (Pits et al. 2008). It might lead to faster colonizing of the stem in shorter plants resulting in higher disease scores. The fact that the frequency of infestation is not significantly increased supports this theory. In contrast to stunting effects caused by azoles, the plant-growth-promotion-effect recorded for the bacterial treatment in glass house experiments by Abuamsha et al (2010a, 2011) might complicate a fast colonization of young seedlings by *V. longisporum*. However, at which developmental stage the plant is infected with *V. longisporum* was not exactly recorded. The first visible symptoms (yellowing of one side of the leaf) appeared in spring. According to Holtschulte (1992) and Paul (1988) young seedlings can be infested after two month in the field. At this time bacterial densities were comparatively high, while possible later infections might have occurred at moments of low HRO-48 density.

Results against *P. brassicae* revealed that HRO-C48 is not capable of reducing the infection with this fungal pathogen under natural conditions. As at Walkendorf and Birkenmoor high bacterial densities were recorded in the rhizosphere during the occurrence of *P. brassicae* (Fig.53), insufficient numbers of bacterial cells as explanation for the absence of effects against the clubroot disease can be excluded. The influence of the randomized position of the plots on the infestation frequency of single repetitions is superior to the influence of treatments. *P. brassicae* is known for its patchy distribution in fields. The enduring spores are spread through cultivation practice.

Hammoudi (2007) found that rhizosphere concentrations of *S. plymuthica* HRO-C48 of more than $\log_{10}$ 5 CFU g^{-1} rhizosphere are necessary to cause control effects against some of the oil seed rape pathogens. In general, HRO-C48 was able to establish at all sites in all three periods of investigation, but bacterial densities fluctuated severely during spring and in most cases the minimum effect level of the bacterial concentration was not reached. These results are contradistinctive to Hammoudi´s field trial data, which indicted constantly higher bacterial densities at Hohenschulen during 2006/07. Strong fluctuation in population dynamics of HRO-C48 might be the reason for temporarily bad performances of the bacteria, especially in spring. Although significant effects on *P. lingam* leaf infestation were recorded in Walkendorf 2008/09, crown canker in the single bacterial treatment was unfortunately more severe compared to the control and V2 in Walkendorf 2008/09 despite high bacterial densities in spring/summer. Without doubt the bacterial density of the rhizosphere is of major importance. However results like these recorded in Walkendorf cause uncertainly of a direct influence of bacterial densities on efficiency.

Population dynamics are influenced by biotic and abiotic factors. With an optimum growth temperature for *S. plymuthica* of more than 20°C (Abuamsha et al. 2010b) declining densities in autumn and winter can be expected. Winter temperatures recorded during 2008 until 2011 were comparatively low, which could have been a major factor for the low survival of the antagonist. However, highest bacterial densities were found in soils of Walkendorf, the field trial site with the highest sand content among all sites, whereas the nutrient-rich marshland of Sönke-Nissen-Koog offered detrimental conditions for survival. HRO-C48 originates from the rhizosphere of rapeseed near Rostock, which is near to Walkendorf. Possibly, the bacterium is adapted to soil conditions prevailing in that region.

Applying living organisms might possibly affect the natural composition of prevailing micro organisms. Research results including *S. plymuthica* HRO-C48 revealed that effects of introduction of these bacteria into the soil microflora were exclusively temporarily and changes in the microbial species population were negligible (Thirup 2003, Blouin-Bankhead 2004). Walsh (2003) found out that even significant changes in the microbial diversity must not necessarily have ecological impacts. Considering bacterial densities reported during these studies, low amounts seem to be a bigger problem than persistence of the introduced bacteria.

Effects of treatments on yields were more pronounced in 2008/09 than in the other trial years. Besides reduction of *P. lingam* symptoms, stunting caused by azoles resulting in higher winter hardiness and tillering (Pits et al. 2008) as well as the growth promoting effect

induced by *S. plymuthica* (Abuamsha et al. 2010a, 2011) could have caused higher yields. The average increase through single treatments was between 1 dt and 1.5 dt by the combination of bacteria and fungicides in this season. This increase in yields could not compensate for costs related with application of fungicides. Even assuming a high price of 45 Euro dt^{-1} rapeseed, fungicide applications were cost-effective only in 5 out of 20 cases (V1 vs. V2; 2008-2011).

This calculation did not consider losses caused by fungicide application, which were mostly occurring in 2010/11. Strikingly, all treatments resulted in higher infestation at root collars and stems and partly lower yields in 2010/11, when crown canker had its strongest impact. In 2010, late sowing and cold climate caused weak development of the young seedlings in Hohenschulen, Hohenlieth, Sartjendorf and Braunschweig, while the fungicide application in autumn even had negative consequences (stress symptoms) in Sartjendorf. Besides their antifungal activity Folicur® and Caramba® influence the growth of plants by modifying their hormonal balance (Rademacher 2000). Probably retarded development of the plants could not match stress induced by the application of the fungicide. Apart from intense crown canker, low yields in the last trial season were primarily related to sub-optimal weather conditions. After snow had melted in January 2011, black frost and game bite caused serious damage to plants at most of the sites followed by longer dry periods in spring. In Barlt, no fungicide was applied in autumn 2010 due to the wet soil conditions. Considering yields of 2011, it was the only site where fungicidal treatment (spring) led to higher yields except for Hohenschulen. On the other hand, the application of fungicides caused thicker stems in spring 2011, what might have distorted the parameter of the pycnidiospores number due to physical reasons. Although fungicide treated plants were firmer and more densely stocked in the last season, application of fungicides led to a decrease in yield in 3 out of 6 cases. As costs related to the bacterial treatment of seed have not yet been assessed, no information can be given about economic benefit of bio-priming HRO-C48.

Any attempts to relate yields with infestation with *P. lingam* should be avoided as sampling was not done in the same plots (except for Barlt and Sönke-Nissen-Koog) that had been harvested and other pathogens -especially *V. longisporum*- could have had influences as well. Due to its morphology, rapeseed is able to compensate damage caused by biotic and abiotic factors, which makes assessment of effects of treatments more difficult (Becker and Leithold 2007), particularly when weather conditions have a major influence on plant development. Taking high variability within the repetitions into account, interpretations on tendencies should be done with much care. However, some significant effects have been reported

despite the biology of the crop and important impact of weather conditions. Under these aspects it can be concluded that *S. plymuthica* HRO-C48 and Azol fungicides have potential to control *P. lingam* leaf infestation. However, application of azoles in autumn and spring as well as the bio-priming of seeds with *S. plymuthica* HRO-C48 might not be cost effective. Despite some high efficiency recorded in bacterial treated variants, results were not significant in most cases, probably because of the separated analyses caused by the correlation of the influencing variables and the high variance within data.

As *S. plymuthica* is a soil-bound bacterium, we hypothesize that induction of resistance mechanisms in the plant plays a major role in the control of *P. lingam,* because a direct contact between mycelium or spores of the disease and the antagonistic bacterium is absent. *S. plymuthica* strain RIGC4 stimulated defence reactions in cucumber seedlings that had been inoculated with *Pythium ultimum* (Benhamou et al. 2000), while strain IC1270 induced resistance to *Botrytis cinerea* on tomato and bean leaves as well as against *Colletotrichum lindemuthianum* on bean (De Vleeschauwer and Höfte 2007). According to Pang et al. (2009) strain HRO-C48 had a control effect against *Pythium aphanidermatum* in cucumber and was able to induce systemic resistance in *Botrytis cinerea* treated beans and tomatoes.

It would nevertheless be advantageous to carry out further field trials, because weather conditions during the period of investigation included non typical winters with exceptionally low temperature and black frost. Compared to artificial infections in glasshouse trials, it is much more challenging to obtain significant effects under outdoor environmental conditions. Another possibility to reduce variability of results might be the use of different sampling methods. Since variance within one plot can be eminent, extended sample numbers along with less sampling dates could provide more homogenous data. This would enable the use of other statistical models.

4.2 Glasshouse trials

In glasshouse trials the effect of *S. plymuthica* HRO-C48 was tested against *V. longisporum*, *P. brassicae* and *S. sclerotiorum*. The investigation on the effect on *V. longisporum* was motivated by the results of Abuamsha et al. (2011) who found that the significant reduction of the disease severity and frequency of infestation with *V. longisporum* was depending on the cultivar. Thus a genetic background of the cultivar seemed to be responsible for the interaction with the bacterial antagonist. Abuamsha et al. (2011) did not include the variety Visby. Consequently, the investigation wanted to assess the interaction of this cultivar with the bacterium and compare it with the most resistant cultivar Trabant. Other cultivars

provided by KWS were included into the trials. In contrast to Abuamsha et al. (2011), this investigation could not detect a control effect of HRO-C48 against *V. longisporum* under given experimental design. The effect of the contamination of the soil with microsclerotia was clearly visible resulting in stunting and a lower number of leaves compared to the healthy control (Fig. 58). Abuamsha et al. (2011) reported a growth promoting effect of the antagonistic bacteria. However, this could not be documented during this investigation. No matter whether treated or not treated with the antagonist, HRO-C48 was unable to compensate the stunting-effect through the growth promoting ability. Compared to Abuamsha et al. (2011) these experiments were conducted with higher inoculum densities, containing about twice as much microsclerotia. Using the inoculum ratio 20:5:1 (soil/vermiculite/*V. longisporum* infested vermiculite) Abuamsha et al. (2011) obtained 60 % infected plants in untreated controls, a frequency of infection also reported from fields without artificial infection. Using the ratio according to Abuamsha et al. (2011) an infestation frequency of less than 10 % was recorded in this investigation, why the ratio of 8:3:1was used to increase the frequency of plant infection. However, even under these conditions no major differences in tolerance were recorded. In this trials Trabant was less infected and had a significant lower AUPDC compared to KWS63, no matter, whether treated with HRO-C48 or not. Higher inoculum densities of *V. longisporum* could be a possible explanation for the lack of effect of the bacterium in these glasshouse trials.

One of the important problems related to increasing percentage of in oilseed rape in the rotation is the Oomycete *Plasmodiophora brassicae*. Consequently, an investigation on possible control effects by the bacterial antagonists was of major interest. In contrast to *P. lingam* and *S. sclerotiorum*, the bacteria and *P. brassicae* zoospores are in direct contact during infection of the plant. Theoretically, an effect on resting spores of HRO-C48 through the production of chitinases is possible as the spores are composed of 25 % chitin, 34 % protein and 18 % lipids, (Moxham and Buczacki 1983) The glasshouse trial carried out by the DLR Rheinland-Pfalz revealed slight effects of the bacterium at low spore concentrations ($\log_{10}$ 3 spores ml^{-1}) that might be related to the production of antifungal metabolites. At higher spore concentration no control effects of HRO-C48 were observed. According to Voorrips (1996) concentrations of $\log_{10}$ 4 - $\log_{10}$ 5 spores $plant^{-1}$ resulted in a nearly entire infestation, while $\log_{10}$ 7 spores resulted in an infestation frequency of 100%.

Glasshouse trials conducted by the DLR led to higher infestation levels at the same spore levels used in glasshouse experiments at Kiel University. A possible explanation might be the use of different *P. brassicae* isolates. According to Voorrips (1995) populations from

field notably vary regarding their virulence and are of no genetic uniformity (Haji Tinggal 1981). This variation was impressively demonstrated by employment of single-spore-isolates. Jones et al. (1982) produced a single-spore-isolate that was virulent to a “resistant” genotype of the host. Crute and Pink (1989) described emergence of higher virulent genotypes through selection pressure, while pathogenicity of a population can also be reduced through the abundant occurrence of susceptible host plants (Seaman et al. 1963). Consequently, the reason for divergent results can have been caused by differences in virulence of *P. brassicae* isolates. In all experiments the cultivar Mendel strikingly demonstrated its resistance even at higher spore levels.

Another major disease of oilseed rape is *Sclerotinia sclerotiorum,* which infects the stem of the plant with ascospores during blossom. The possible induction of resistance against this disease by HRO-C48 was therefore also investigated. The glasshouse trials revealed a significant reduction in size of lesions caused by *S. sclerotiorum*. In absolute terms this reduction was small, but results of field trials (not shown) indicated a possible control effect even under natural conditions. Fernando et al. (2006) found that application of *Pseudomonas chlororaphis* on petals of rapeseed provided good control effects against ascospores of *S. sclerotiorum,* while Kamensky et al. (2002) reduced white mold on cucumber leaves by foliar application of *S. plymuthica* IC 14. These investigations tested direct antagonistic abilities of bacteria spayed on the canopy, while the presented investigation produced trial set-ups enabling the examination of indirect effects. Due to limited survival of Gram-negative bacteria on the canopy and the availability of effective fungicides, applications of these bacteria have little potential for practical use. Therefore, this investigation only targeted at assessing the effect of the bio-priming of the seeds. As with *P. lingam,* no direct contact between infested petals and the antagonist is possible if *S. plymuthica* is applied to seeds, as this bacterium has no endophytic potential (Abuamsha et al. 2010a). Probably, mode of action is induction of resistance. According to Hammerschmidt and Kuc (1995) an interval of 3-7 days is needed for this effect. Since no cultivars resistant to *S. sclerotiorum* are available at the moment, bio-priming with HRO-C48 might represent an alternative to chemical control. Further field trials have to confirm the ability of the antagonist to reduce *S. sclerotiorum* severity.

4.3 Seed treatment

The key to successful colonisation of the rhizosphere by antagonistic bacteria is an efficient seed treatment procedure. Müller and Berg (2008) reported that bacterial densities of HRO-

C48 of $\log_{10}$ 3 CFU seed^{-1} are capable to colonize the rhizosphere of rapeseed seedlings in a controlled environment. Independent from the bacterial concentration in the seeds ($\log_{10}$ 3- 7) the resulting density in the rhizophere was $\log_{10}$ 5 CFU g^{-1} isolated from 30 days old plants. Supposing that control effects of the antagonist are dependent on the density of the antagonist in the rhizosphere, results by Hammoudi (2007) need to be taken into consideration, who report a minimum effect level of a bacterial density of $\log_{10}$ 5 CFU seed^{-1} for control of *P. lingam*. However, under field conditions (compared to controlled glasshouse conditions) bacteria are exposed to abiotic and biotic factors that might complicate a sustainable colonization of the rhizosphere. Therefore, higher concentrations of the antagonist inside the seeds can still have advantages of lower numbers.

In order to improve the seed bio-priming procedure a spray system was tested. Seeds treated with the airbrush gun contained similar bacterial densities compared to bio-primed seeds of Abuamsha et al. (2010b) after storage of 30 days at 4° or 20°C. In contrast, Müller and Berg (2008) found cell densities of bio-primed seeds of $\log_{10}$ 5 CFU seed^{-1} regardless of the storage temperature. The difference between closed and opened storage in the current trial setup was the water-impermeable Parafilm®. While an air exchange is possible, moisture is kept inside the Petri dishes. In most cases, closed storage resulted in higher bacterial concentrations, but probably due to the limited number of cases, varieties were not significantly different. In contrast, closed storage promotes the formation of mould and untimely germination. Abuamsha et al. (2010b) also analysed the survival of bacteria in closed Eppendorf tubes that allowed no air exchange. This type of storing intensely prolonged the survival of bacteria resulting in densities of >$\log_{10}$ 5 CFU seed^{-1} after 11 month when stored at 4°C compared to an unsealed storage. In practise seeds are filled into paper sacks after chemical treatment which are stored at 10°C and reach final consumers within six weeks. As paper sacks allow the exchange of moisture and air, seeds ought to be stored in high barrier plastic bags allowing no gas exchanges to ensure adequate bacterial densities for longer times.

Drying of the seeds and higher initial bacterial concentrations did not have the expected effect. The original idea was to accelerate and increase the uptake by the seeds due to lower water content. Another objective was to produce seeds with lower seed moisture after treatment. In fact, bacterial densities of dried seeds were lower in most cases compared to the samples containing moisture of 5.8 %, while treatments with higher initial concentrations ($\geq\log_{10}$ 11 CFU ml^{-1}) led to slightly increased densities in average that can hardly justify the additive labour for achievement of higher bacterial densities. In contrast to the concentration,

the relation of applied bacterial suspension to the amount of seeds was of importance. Since both factors refer to the applied amount of bacteria, applied water amount is the determining parameter. A specific amount of water is necessary to induce bulging of the seeds that leads to imbibtion of the bacteria. Though the total amount of applied bacteria was higher in cases of a $\geq\log_{10}$ 11 CFU ml^{-1}initial concentration and the ratio 1:3 compared to $\geq\log_{10}$ 10 CFU ml^{-1} and the ratio 1:1, cell densities were increased implying ratio 1:1.

Spray application technique represents a timesaving alternative to bio-priming but due to high seed moisture subsequent chemical coating is not possible. Based on physical reasons applied water amount cannot be reduced below a minimum and following re-drying or storage is necessary.

Bacterial enrichment of talcum, vermiculite and diafill was successful. The clay minerals had to be re-dried to recover their ability of binding excessive liquid, but re-drying logically led to decreased bacterial densities. Optimal drying time of two hours regarding bacterial and water content resulted in sufficient bacterial densities of $\log_{10}$ 7 CFU g^{-1} - $\log_{10}$ 8 CFU g^{-1} after two days. Storage temperature affected the survival of the bacteria inside the clay minerals as expected. As it should not be a problem to enrich the clay minerals immediately before applying them in the chemical priming process, storage ability was not of major importance. Anyway, non re-dried clay minerals revealed bacterial amounts of $\log_{10}$ 5 CFU g^{-1} - $\log_{10}$ 7 CFU g^{-1} after 30 days of storage (data not shown).

The procedure of bacterial enrichment used in this trial originates from Vidhyasekaran and Muthamilan (1995), who enriched different clay minerals with the gram negative bacterium *Pseudomonas fluorescens.* While initial concentration were comparable with results of the presented investigation, storage in sealed polythene bags (25°C) led to *P. fluorescens* concentrations of $\log_{10}$ 7 in talcum and $\log_{10}$ 6 in vermiculite. One kilogram of chickpea seeds was treated with only 5 g of the enriched clay minerals and 30 days post-planting rhizosphere concentrations of $>\log_{10}$ 4 CFU g^{-1} were achieved.

In the current study, no bacteria could be re-isolated from rapeseed seeds that were treated with *S. plymuthica* enriched clay minerals within the process of chemical coating. The detection limit of applied re-isolation method is $\log_{10}$ 2 $seed^{-1}$. Assuming clay mineral had bacterial densities of $\log_{10}$ 8 g^{-1} at coating and 25 g of the enriched binder were used treating one kilogram of seeds (thousand seed weight: 6 g), 1.6 $\log_{10}$ 4 cells would be available for every single seed under an ideal distribution. Visual observations revealed that seeds seemed evenly coated and there were no residues of the clay minerals inside the drum. In previous

trials, HRO-C48 was grown in TSB media enriched with different concentrations of the agents used for coating, showing no negative impact of chemicals applied to the seeds. Bacteria might have died during the process of desiccation and occurring shear forces. Further unequal distribution of the bacteria in the clay minerals could have led to bacterial level below detection limit.

As the bacterial enrichment of the clay minerals was limited due to insufficient moisture content, there were hardly any options to optimize the application of this procedure. Bacterial survival during chemical coating might possibly be enhanced by addition of protective agents like trehalose (Strøm 1998). Due to physical reason there is no possibility to realize seeds containing $\log_{10}$ 6 CFU without increasing the amount of applied clay minerals.

5. Summary

The efficiency of a seed treatment of oilseed rape (*Brassica napus*) with the antagonistic rhizobacterium *Serratia plymuthica* (strain HRO-C48) against blackleg disease caused by *Phoma lingam, Verticillium longisporum* and *Plasmodiophora brassicae* was tested separately and in combination with azole fungicides in perennial field trials at nine sites in northern Germany. Furthermore, the performance of HRO-C48 against *V. longisporum*, *P. brassicae* and *S. sclerotiorum* was examined in glass house trials.

Seeds were enriched with bacteria via bio-priming. The exposure of seeds in bacterial suspensions of $\log_{10}$ 10 CFU ml^{-1} for 6 hours reached densities of $\log_{10}$ 6 CFU $seed^{-1}$. Occurrence and severity of *P. lingam* leaf infestation stem and crown canker was highly variable depending on the year and field site. No correlation between leaf infestation and crown canker infestation was recorded. Reductions of *P. lingam* infestation frequency (6-19%) and leaf pycnidia area under disease progress curves (AUDPC) (17-70 %) provided by the single HRO-C48 treatment were significant in 1 out of 7 cases compared to the untreated control in the first year of trials (2008/09). In combination with fungicides the effect was slightly higher, leading to reductions of infestation frequency (11-26 %) and leaf pycnidia AUDPC (38-78 %), which were significant in 2 out of 7 cases.

In the following years the level of *P. lingam* leaf infestation was lower and there were no significant reductions provided by the single HRO-C48 treatment. Disease score, pycnidia and infestation frequency of weaker infestations with crown canker was controlled significantly by the bacterium and the combined treatment of azol fungicides and bacteria at 3 out of 7 trial sites in the first season, whereas the single fungicidal treatment could not provide significant reductions. More severe crown canker infestations recorded during the last season of trials (2010/11) were not reduced by HRO-C48, whereas fungicide treatment even enhanced the severity in 6 out of 7 cases in this trial year. *P. lingam* stem infestation was not severe during the complete investigation period. Neither Azoles nor *S. plymuthica* effected the development of these disease symptoms considerably.

Treatments led to increased yields in 2008/09, whereby the combined treatment of bacterium and azoles resulted in a surplus of 1.5 dt ha^{-1} in average. During the following trial seasons treatments only had minor impact on yield. In general, differences within the yields were not significant.

S. plymuthica was able to colonize the rhizosphere of the rapeseed plants efficiently (log_{10} 6-7 CFU/g rhizosphere) at all trial sites and years. The trial sites Walkendorf and Birkenmoor revealed the highest population densities during the investigation period, while isolation of the antagonist from marshland soils was generally low. The population dynamics were influenced by different soils and climatic conditions and effective bacterial densities during the whole growing period often dropped below the minimum effect level in winter/spring, what might have been the reason for insufficient control effects of the antagonist.

During the investigation period *V. longisporum* naturally occurred in 16 out of 21 field trials leading to infestation frequencies as high as >70 % at several sites. Summarized results for *V. longisporum* control, including all sites and trial years, revealed remote differences within the non-fungicide treated variants, while fungicide treated variants showed an increased infestation compared to the control and a significant higher infestation compared to the single bacterial treatment. Cultivar dependent control effects reported by previous investigations against *V. longisporum* in glass house trials could not be confirmed with the cultivar Visby used for field trails and other cultivars tested also in glasshouse trials. Field and glasshouse trials demonstrated that HRO-C48 is not able to reduce *P. brassicae* infections at common levels of spore concentrations. The resistance of the cultivar Mendel was confirmed. In glasshouse trials the antagonist reduced size of lesions caused by artificial inoculated *Sclerotinia sclerotiorum* significantly.

Attempts to improve the bio-priming with *S. plymuthica* by use of an airbrush gun led to bacterial amounts of >log_{10} 7 CFU $seed^{-1}$. Treated seeds revealed cell densities of log_{10} 4 – 5 CFU $seed^{-1}$ after 30 days of storage at 4 °C, which is comparable to the storage ability of bio-primed seeds. Moisture content post-treatment was too high, why subsequent drying was necessary, which reduced the amount of bacteria in the seeds. Clay minerals were successfully enriched with the bacterium resulting in densities of nearly log_{10} 9 CFU g^{-1}, however, bacteria could not be re-isolated from clay mineral treated seeds two days after treatment.

6. Zusammenfassung

In mehrjährigen Freilandversuchen an insgesamt neun Standorten in Norddeutschland wurde die Wirkung einer Saatgutbehandlung mit dem antagonistischen Rhizobakterium *Serratia plymuthica* (Stamm HRO-C48) alleine und in Kombination mit Azol-Fungiziden gegenüber den Rapspathogenen Wurzelhals- und Stängelfäule (*Phoma lingam*), der Rapswelke (*Verticillium longisporum*) und der Kohlhernie (*Plasmodiophora brassicae*) untersucht. Hierfür wurden die Rapssamen vor der Aussaat mittels sechsstündigem Bio-priming in einer *S. plymuthica* Suspension (log_{10} 10 CFU ml^{-1}) mit Bakteriendichten von log_{10} 6 CFU $Samen^{-1}$ angereichert.

Während das Auftreten des Erregers *P. lingam* stark von dem Versuchsstandort und -jahr abhängig war, konnte keine Korrelation zwischen *P. lingam* Blattbefall und Wurzelhalsbefall festgestellt werden. Im ersten Versuchsjahr (2008/09) konnte *S. plymuthica* die „area under disease progress curves“ (AUDPC) des *P.lingam* Blattbefalls um 17-70 % und die der Befallshäufigkeit um 6-19 % reduzieren, wobei die Reduktion an einem von sieben Standorten signifikant war. Mit Reduktionen der Befallshäufigkeit (11-26 %) und Blattpyknidien (38-78 %) AUDPC, die an zwei von sieben Standorten signifikant waren, konnte eine leicht erhöhte Wirkung gegen den Blattbefall in Kombination mit Azol-Fungiziden erzielt werden. In den beiden folgenden Untersuchungszeiträumen war der *P. lingam* Blattbefall schwächer und es gab keine signifikanten Reduktionen durch *S. plymuthica*.

Durch *S. plymuthica* konnten im ersten Versuchsjahr der Befallswert, Pyknidienbesatz und die Befallshäufigkeit von schwächer ausgeprägtem Wurzelhalsbefall signifikant an drei von sieben Standorten reduziert werden, während für alleinige Azol-Applikationen keine signifikanten Unterschiede im Vergleich zur unbehandelten Kontrolle ermittelt wurden. Stärkerer Wurzelhalsbefall, wie er im letzten Versuchsjahr (2010/11) auftrat, konnte nicht durch das antagonistische Bakterium reduziert werden, während ein Fungizideinsatz hier in sechs von sieben Fällen zu einer leichten Erhöhung des Befallswertes führte. Im gesamten Untersuchungszeitraum konnte kein relevanter *P. lingam* Stengelbefall ermittelt werden und es gab keinen nennenswerten Einfluss der verschiedenen Behandlungen auf den Befall.

Alle Behandlungen führten zu gesteigerten Erträgen in der Versuchssaison 2008/09. Durch die kombinierte Anwendung von Azolen und *S. plymuthica* wurde im Durchschnitt ein Mehrertrag von 1,5 dt/ha erzielt. In den beiden folgenden Versuchsjahren hatten die

Behandlungen keinen nennenswerten Einfluss auf den Ertrag. Generell waren Unterschiede zwischen den Erträgen der verschiedenen Varianten nicht signifikant.

S. plymuthica konnte an allen Versuchsstandorten und in allen Versuchsjahren erfolgreich die Rhizosphäre von jungen Rapspflanzen im Herbst besiedeln (log_{10} 6-7 CFU g^{-1} Rhizosphäre). Die höchsten Bakteriendichten wurden an den Standorten Walkendorf und Birkenmoor isoliert, während ermittelte Zellzahlen aus schweren Marschböden in der Regel niedrig waren. Die Populationsdynamik wurde stark durch die klimatischen Bedingungen und den Bodentyp beeinflusst. Dementsprechend sanken die Zellzahlen im Winter/Herbst oftmals unter die kritische Dichte, was ein Grund für eine fehlende Wirkung des Bakteriums gewesen sein könnte.

Ein natürliches und teilweise starkes Auftreten von *V. longisporum* mit Befallshäufigkeiten von über 70 % konnte in 16 von 21 Versuchsfällen verzeichnet werden. Die Analyse der zusammengefassten Freilandergebnisse aller Standorte und Jahre brachte leicht unterschiedliche Befallswerte innerhalb der nicht fungizid-behandelten Varianten hervor, während die Befallswerte der fungizid-behandelten Varianten im Vergleich zur einzelnen Bakterienbehandlung signifikant erhöht waren. Der in der Literatur beschriebene, sortenabhängige Effekt einer Saatgutbehandlung mit *S. plymuthica* auf *V. longisporum*, konnte bei der in den Freilandversuchen verwendeten Sorte Visby und anderen in Gewächshausversuchen getesteten Sorten nicht bestätigt werden. Freiland- und Gewächshausversuche haben ergeben, dass HRO-C48 nicht in der Lage ist, den Befall mit *P. brassicae* zu vermindern, während die Resistenz der Sorte Mendel gegenüber dem Pathogen bestätigt werden konnte. Hingegen wurde eine signifikante Reduktion der durch eine künstliche *Sclerotinia sclerotiorum* Infektion verursachten Läsionen durch das Bakterium nachgewiesen.

Bei Versuchen das Verfahren des Bio-primings durch Applikation von *S. plymuthica* mittels einer Airbrush-Pistole zu verbessern, konnten Rapssamen mit Bakteriendichten von >log_{10} 7 CFU $Samen^{-1}$ angereichert werden. Aus den behandelten Samen konnten nach dreißigtägiger Lagerung bei 4 °C Zelldichten von log_{10} 4-5 CFU $Samen^{-1}$ isoliert werden, was der Lagerstabilität bio-geprimter Samen entspricht. Der hohe Feuchtigkeitsgehalt der Samen nach der Sprühapplikation machte eine anschließende Rücktrocknung unvermeidbar, die wiederum zu einem Absterben der Bakterien führt. Tonminerale konnten mit Dichten von fast log_{10} 9 CFU g^{-1} erfolgreich mit *S. plymuthica* angereichert werden. Dagegen konnten

keine Bakterien nach dem Beizprozess, in dem das angereicherte Tonmineral zum Abbinden der chemischen Flüssigbeize diente, aus den Samen reisoliert werden.

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