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Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of brassicas with an antagonistic strain of *Bacillus subtilis* in Zimbabwe

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Abstract

Biological control efficiency of an antagonistic, endophytic strain of *Bacillus subtilis* (strain BB) was evaluated against three strains of the black rot pathogen, *Xanthomonas campestris* pv. *campestris* (Xcc), in four *Brassica* crops (cabbage, cauliflower, rape and broccoli) grown during three consecutive growing seasons and on two soil types, in two different areas in Zimbabwe. Strain BB controlled the disease caused by strain Xcc B-147 in all *Brassica* crops during the dry and short rainy seasons. A similar effect was observed in cabbage using the strain Xcc 33908. Biological control was effective in broccoli, but not in cabbage and rape during the main rainy season in clay loam soil and limited biological control effect was still observed when these crops were grown in sandy loam soil. The endophytic colonisation of cabbage roots by strain BB was confirmed by immuno-blotting during the whole growing season. Biological control of black rot with strain BB is discussed in relation to its effect on Xcc strains, *Brassica* crops and to the effect of weather and soil conditions.

Introduction

Brassicas are an important source of food in many African countries. In Zimbabwe, brassicas are grown all the year round except in the rainy season when cultivation of brassicas almost becomes impossible due to black rot caused by the bacterial pathogen, *Xanthomonas campestris* pv. *campestris* (Xcc) (Pammel) Dowson. Cabbage, cauliflower, broccoli, Brussels sprouts, collard, rape, kale, kohlrabi, mustard, rutabaga, turnip, watercress and radish are susceptible to black rot (Williams, 1980; Mguni, 1996). Xcc may be seed borne and can infect the plant through roots, wounds, hydathodes and leaf stomata (Schaad and Alvarez, 1993). The typical leaf symptoms of black rot are V-shaped lesions on the leaf margin with black veins. Under favourable conditions of high temperature and humidity, discolouration of the veins reaches the stem and from there the bacteria move systemically up and downwards to other leaves. Infected leaves may fall off prematurely and soft rot caused by *Erwinia* spp. and *Pseudomonas* spp. may set in (Williams, 1980).

Infected seeds are an important source of black rot in Zimbabwe, and the use of pathogen free seeds and resistant varieties have been recommended for managing the disease (Mguni et al., 1999). Crop rotation and control of cruciferous weeds are also good cultural practices (Williams, 1980; Schaad and Alvarez, 1993). Despite these procedures, black rot remains the most important disease of crucifers worldwide (Williams, 1980; Mguni, 1996).

Biological control of plant pathogens using antagonistic bacteria is a promising strategy for plant protection (Kloepper et al., 1999). While most of the

studies conducted with bacterial biocontrol agents have involved organisms which colonise externally roots and leaves, in recent years, bacteria that colonise internal plant tissues have received more attention (Chen et al., 1995; Pleban et al., 1995; Mguni, 1996; Assis et al., 1998; Wilhelm et al., 1998). Internal colonisation of plants by non-pathogenic bacteria seems to be a natural widespread phenomenon since endophytes have been isolated from healthy plant tissues of various plant species (Hollis, 1951; Samish et al., 1963). One of the advantages of using endophytes is that, once inside the host, they are better protected against environmental stress and microbial competition (Law and Lewis, 1983). Thus, endophytes are not exposed to UV radiation and fluctuations of temperature and moisture compared to phylloplane bacteria (Chen et al., 1995). Strains of *Bacillus* are among the most common bacteria found to colonise plants endophytically (Lilley et al., 1996; Mahaffee and Kloepper, 1997) and members of this genus are known to produce metabolites that affect bacterial and fungal growth (Loeffler et al., 1986; Krebs et al., 1998). Furthermore, the use of endophytic *Bacillus* strains as biological control candidates has the advantage that they form endospores that can easily be formulated and stored. Mguni (1996) isolated several *Bacillus* strains from *Brassica* species with good antagonistic potential against black rot, including *B. subtilis*.

The aims of the present work were (i) to investigate the ability of *B. subtilis* (strain BB) to control black rot under different growing seasons, on four different brassica crop plants and using three different Xcc strains, (ii) to compare the ability of BB to control black rot in brassicas growing in two different soil types in two different areas and (iii) to examine the establishment and persistence of the bacterium associated with roots and stems of cabbage under field conditions.

Materials and methods

Microbial and plant materials

Bacterial strains. *Bacillus subtilis* strain BB was isolated from *Brassica* seeds by Mguni (1996). Xcc, strain B-147, was isolated from cabbage in Hawaii by A.M. Alvarez, University of Hawaii. Xcc strains 33908 and 33907 were isolated from commercial seeds from Zimbabwe by Mguni (1996). All strains were kept at −80 °C in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) amended with glycerol (20%).

Seed disinfection. Seeds were certified as free from Xcc. However, they were surface disinfected to avoid the presence of any pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 1 min in 75% ethanol, immersing for 3 min in 1–2% sodium hypochlorite and washing four times in sterile distilled water. Seeds were left to dry overnight in the flow cabinet. Sterility control was performed after seed disinfection by plating 100 seeds per disinfected seed lot on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) and incubating at 25 °C. If no microbial growth was detected on the plates, the seed samples were considered sterile and used in further experiments.

Plant material. Seedlings of *Brassica* were raised at a commercial plant nursery in Zimbabwe. Seeds were sown in multi-pots containing composted pine bark. In the nursery, mono ammonium phosphate (6.0 g per 5 l of water), potassium nitrate (6.5 g per 5 l of water) and ammonium nitrate (5.0 g per 5 l of water) were applied three times a week during the first month of growth. Watering was done twice a day using the Microjet sprinkler irrigation system.

Preparation of the bacterial inoculum. Twenty millilitre of a sterile saline solution (0.85% NaCl) were added to the Petri dishes containing 24-h-old bacterial cultures growing on TSA (Difco Laboratories, Detroit, MI, USA). The cultures were scraped with a glass rod and the suspensions homogenised by agitation in a Vortex mixer. The amount of inoculum was measured in a spectrophotometer and adjusted with sterile saline solution ($OD_{600} = 0.1$ was equivalent to 1×10^8 colony forming units (CFU)/ml).

Field experiments

Field location. The field experiments 1–3, 5 and 6 were conducted at Henderson Research Station, Mazowe, Zimbabwe. The soil type at Henderson is red clay loam. Field experiment 4 was conducted at the Horticultural Research Station, Marondera, Zimbabwe. The soil at Marondera is sandy loam. The amount of rainfall during the conduction of the biocontrol experiments is presented in Table 1. Temperature conditions were not much variable during the experimental period. The maximum average temperature ranged from 24.4 to 30.27 °C (day temperature), while the minimum varied between 5.55 and 15.85 °C (night temperature).

Table 1. Biocontrol effect of *B. subtilis* (strain BB) against Xcc on different brassicas, sites and seasons in field experiments

Experiment	Growing period and site[1]	Total rainfall (mm)	Plant	Inoculation[2]	EBRindex[3]	IBRindex[4]
1	4–7/98 Mazowe	0	Cabbage[5]	Control	0.00 *c*	0.00
				BB	0.02 *c*	0.00
				Xcc (B147)	0.22 *a*	0.00
				BB + Xcc	0.10 *b*	0.00
2	9–12/98 Mazowe	432	Cabbage[5]	Control	0.00 *b*	0.08 *b*
				BB	0.01 *b*	0.08 *b*
				Xcc (B147)	0.16 *a*	0.38 *a*
				BB + Xcc	0.04 *b*	0.12 *b*
3	11/98–2/99 Mazowe	1190	Cabbage[6]	Xcc (B147)	Destroyed	Destroyed
				BB + Xcc		
			Rape[7]	Xcc (B147)	0.86 *a*	0.00
				BB + Xcc	0.84 *a*	0.00
			Broccoli[8]	Xcc (B147)	1.05 *a*	0.00
				BB + Xcc	0.25 *b*	0.00
4	11/98–2/99 Mazowe	1113	Cabbage[6]	Xcc (B147)	2.21 *a*	0.00 *a*
				BB + Xcc	1.95 *b*	0.05 *a*
			Rape[7]	Xcc (B147)	0.96 *a*	0.00
				BB + Xcc	0.72 *b*	0.00
			Broccoli[8]	Xcc (B147)	0.92 *a*	0.00
				BB + Xcc	0.20 *b*	0.00
5	4–7/99 Mazowe	0	Cabbage[9]	Xcc (B147)	0.25 *a*	0.94 *a*
				BB + Xcc	0.09 *b*	0.19 *b*
			Cauliflower[10]	Xcc (B147)	0.30 *a*	0.15 *b*
				BB + Xcc	0.14 *a*	0.09 *a*
			Broccoli[8]	Xcc (B147)	0.15 *a*	0.06 *a*
				BB + Xcc	0.01 *b*	0.06 *a*
6	9–11/99 Mazowe	373	Cabbage[5]	Xcc (B147)	0.89 *a*	0.40 *a*
				BB + Xcc	0.78 *b*	0.18 *b*
				Xcc (B147)	0.91 *a*	0.45 *a*
				BB + Xcc	0.78 *b*	0.16 *b*
				Xcc (B147)	1.03 *a*	0.26 *a*
				BB + Xcc	1.04 *a*	0.28 *a*

[1]Sites in Zimbabwe, Mazowe (Henderson Research Station, clay loam soil) and Marondera (Horticultural Research Station, sandy loam soil); [2]Xcc for *Xanthamonas campestris* pv. *campestris*, BB for *Bacillus subtilis*; [3]EBRindex for external black rot index based on symptoms on the leaves; [4]IBRindex for internal black rot index based on symptoms in the heads; [5]cv. Copenhagen Market; [6]cv. Hercules; [7]cv. Giant English; [8]cv. Green Valiant; [9]cv. Sugar Loaf; [10]cv. TH25. Means are compared within treatments in the same experiment by the Newman–Keuls test at 5%. Number of replicates per treatment is 4.

Cultural practices. One day before transplantation of the seedlings, a basal fertiliser (6 : 12 : 6) at the rate of 1000 kg per ha was applied to the soil. Twenty-one and forty-two days after transplantation, the plots were top-dressed with ammonium nitrate (34.5% N) at the rate of 100 kg per ha.

Plants at the Henderson Research Station and the Horticultural Research Station were grown under overhead irrigation conditions. Plants were generally irrigated three times a week (3 h per day, 25 mm of water per h) during the dry season. During the rainy season, in the absence of natural rainfall, plants were watered whenever it was necessary.

Inoculation. Experiments 1 and 2 were performed to examine the ability of BB in controlling black rot and to follow the population of the antagonist in the plant (experiment 2). In these experiments, 1-month-old cabbage seedlings were lifted from their pots and the roots were carefully washed with tap water to eliminate most of the soil. The root tips were cut (0.5 cm) with a pair of scissors and the plant roots were immersed for 2 h in the inoculum suspension of the antagonist (5×10^8 CFU/ml). One week after inoculation with the antagonist, the pathogen was applied to plots to be treated with Xcc by spraying 2 ml of inoculum suspension (1×10^8 CFU/ml) per plant late

in the afternoon. Three different controls were used: (a) plants only sprayed with sterile water (control), (b) plants only inoculated with the antagonist (BB) and (c) plants only inoculated with the pathogen (Xcc).

Plants were inoculated without cutting the root tips in experiments 3–5. In these experiments, the biocontrol effect of BB against Xcc was measured in three different *Brassica* crops and two different sites in Zimbabwe (experiments 3 and 4). One-month-old *Brassica* seedlings were lifted from the pots and their roots were dipped into the inoculum suspension (1×10^8 CFU/ml), containing the antagonist, before planting in the field. Roots of infested control plants (to be inoculated with Xcc 1 week later) were dipped in sterile saline solution before transplantation. Infested control consisted of inoculation with the pathogen alone. In these experiments, the pathogen was sprayed in leaves of the guard row plants (one leaf/plant) that were wounded by cutting 1 cm off the youngest leaf. The leaves were sprayed until runoff with 2 ml of pathogen suspension (1×10^8 CFU/ml) per plant. The inoculation of guard rows with the pathogen in these experiments was to assure a homogenous distribution of the inoculum in the experimental plots. Experiments 3 and 4 were conducted in the rainy period where the inoculum pressure of the pathogen was high.

Experiment 6 was conducted in order to measure the biocontrol effect of BB against three different strains of Xcc on cabbage. Seedlings were inoculated similarly to experiments 1 and 2, but root washing was not conducted in this experiment to reduce plant stress. The root tips were instead removed and inoculated as described in experiments 3–5. After inoculation, the seedlings were planted in the field. Also, in this experiment, besides inoculation with the antagonist at planting, plants were re-inoculated 1 and 2 months after the first inoculation with 1 ml of BB (5×10^8 CFU/ml) per plant applied to the soil close to the main root.

Assessment of the biocontrol effect. The biological control effect in experiments 1–6 was quantified by assessing external and internal black rot symptoms, 90 days after transplantation. Broccoli plants in experiments 3 and 4 were assessed, 75 days after transplantation, just before flowering. Experiment 6 was assessed 70 days after transplantation.

For scoring the external black rot index (EBRindex), leaves not forming part of the head were examined for black rot symptoms and assessed as follows:

$$\text{EBRindex} = \frac{0a + 1b + 2c + 3d + 4e}{T}$$

where: 0, 1, 2, 3 and 4 indicate respectively none, >0–10%, 11–20%, 21–30% and >30% of the surface of a leaf showing black rot symptoms; *a–e* correspond to the number of leaves in the infection category; *T* is the total number of external leaves.

For assessing the IBRindex, cabbage, broccoli and cauliflower heads were cut perpendicularly into quarters and the internal symptoms were assessed as follows: 0 = No discolouration, no symptoms on the heart leaves (healthy plants); 1 = vein discolouration extends $<\frac{1}{2}$ of the stem, no symptoms on the heart leaves; 2 = vein discolouration extends $>\frac{1}{2}$ of the stem, no symptoms on the heart leaves; 3 = vein discolouration of stem and 1–3 of the heart leaves and 4 = vein discolouration of stem and on more than 3 heart leaves.

Experimental design and statistical analysis. All experiments were laid out in a complete randomised block design with four replicates. The plots in experiment 1 contained 40 plants (4 rows of 10 plants each). The plants were transplanted 45 cm apart within the row and 60 cm between the rows. Experiment 2 was conducted with plots containing 80 plants per plots (8 rows of 10 plants each) where part of the plants was sampled for population studies. Experiments 3–6 contained plots with 40 plants (8 rows with 5 plants). The distance between the rows in these experiments was 60 cm and within the rows was 40–35 cm. In all experiments, plots were surrounded by a guard row of the test plants.

Analysis of variance for biocontrol experiments were conducted using the General Linear Models (GLM) of the Statistical Analysis Systems (SAS) package (Statistical Analysis Systems Institute Inc., Cary, NC, USA). The experiments were analysed as a completely randomised block using four blocks with 10 plants per treatment in experiment 1 and 3, and 20 plants per treatment in experiments 2, 4, 5 and 6. Means of each treatment in biocontrol experiments were compared using the Student Newman–Keuls test ($P < 0.05$).

Population studies

Re-isolation of BB from field growing plants. Part of the plants belonging to experiment 2 (control and

treated with BB) were collected 15, 30, 60 and 90 days after inoculation with BB and used for population studies. Root (2 cm cut from the upper root part) and stem (1 cm cut from the lower stem part) segments from inoculated (BB) and uninoculated plants (control) were thoroughly washed with tap water, cut with a scalpel and transferred for 2 min to Petri dishes containing 15 ml of 1–2% sodium hypochlorite. Samples were then washed three times consecutively in sterile distilled water, placed into plastic bags containing 2–8 ml of sterile saline (0.85%) amended with glycerol (20%) and then crushed with a hammer. One millilitre of the plant extract was transferred to a sterile vial and kept in the freezer at $-20\,^{\circ}$C until drop platting was performed. To assure that the sections were completely surface disinfected 100 µl of the last wash was transferred to TSB and incubated at 27 °C. If contamination was detected, the sample was discarded.

Bacterial population was detected using drop plating combined with colony blotting. Before conducting drop plating, plant extracts were serially diluted down to 10^{-4}. Petri dishes containing TSBAS were divided into four sections (one section to each dilution factor). Five drops (10 µl drop) of each dilution factor were transferred to their respective sections on the plate. TSBAS was prepared with 15 g Bacto agar (Difco Laboratories, Detroit, MI, USA), 1.5 g TSB, 50 g NaCl and 1000 ml dH_2O. After sterilisation, cycloheximide (10 ppm, Sigma Chemical, St. Louis, MO, USA) and 2,3,5-triphenyl-tetrazolium chloride (10 ppm, Merck, Darmstadt, Germany) were added to the medium. The plates were left to dry in the flow cabinet until the agar had absorbed the drops and then incubated at 30 °C.

Quantification of BB population by colony blotting. Three days after incubation, the colonies were transferred to nitrocellulose filters by placing the filter on the agar. The filters were let on the agar until they were completely soaked. Subsequently, they were transferred (colony side up) to a Petri dish containing 10 ml immuno-blocking buffer {50 mM Tris (pH 10.2), 150 mM NaCl, 2% Tween 20 and 0.5% bovin serum albumin} and incubated for 20 min with gentle shaking. The filters were afterwards washed three times (3×10 ml) with immuno-washing buffer {50 mM Tris (pH 10.2), 150 mM NaCl, 2% Tween 20 and 0.05% bovin serum albumin} for 5 min (each washing) and incubated overnight with the primary antibody (Wulff, 2000) diluted 50.000 times. Next day, the filters were washed again (3×10 ml) with the immuno-washing buffer for 5 min and incubated with gentle shaking for 2 h with the secondary antibody (anti-chicken alkalic phosphatase, Sigma Chemical, St. Louis, MO, USA), diluted 50.000 times. After 2 h incubation, the filters were washed three times again with immuno-washing buffer and then transferred to 10 ml staining solution composed of 100 mM ethanolamine (pH 9.6), 5 mM $MgCl_2$, 0.17 mg/ml bromo-chloro-indolyl phosphate (Sigma Chemical, St. Louis, MO, USA) and 0.33 g/ml nitroblue tetrazolium (Sigma Chemical, St. Louis, MO, USA). After 15 min incubation in the dark, the staining reaction was stopped with water. Violet spots on the filters corresponded to colonies of BB on the plates.

Experimental design and statistical analysis. The experimental design used was the same as described in field experiment 2. The obtained data was transformed to Log of CFU per g fresh weight, since transformation reduced the variation compared to the untransformed data. Samples where no bacteria were detected were considered as zero, and included in the calculation of the means. The experiment was conducted with four blocks with nine replicates per block. Standard deviations and analysis of variances were conducted using respectively the programs GraphPad Prism (GraphPad Software, San Diego, USA) and GLM of the SAS package (Statistical Analysis Systems Institute Inc., Cary, NC, USA). Means were compared using the Student Newman–Keuls test ($P < 0.05$).

Results

Field experiments

In experiment 1, performed during the dry season, April–July 1998, strain BB significantly reduced the amount of black rot symptoms on cabbage leaves (EBRindex) when co-inoculated with the pathogen compared to the plants only treated with the pathogen (Table 1). Almost no black rot symptoms were seen in plants inoculated only with BB and no symptoms were observed in control plants. At harvest, no internal black rot symptoms were observed in the cabbage heads (Table 1).

In experiment 2, conducted during the short rainy season from September–December 1998, BB was able to reduce the attack of black rot on cabbage leaves (EBRindex) compared to plants treated with the pathogen alone (Table 1). Considering the internal black rot symptoms (IBRindex), control plants, plants

treated with BB alone and plants treated with BB + Xcc showed significantly less symptoms compared to plants treated with the pathogen only (Table 1).

Experiment 3 was conducted during the full rainy season (November 1998–February 1999) and in the red clay loam soil (Table 1). At that time of the year, cabbage was more susceptible than rape and broccoli. The heads were totally rotten at harvest (90 days after transplanting). Thus, the assessment of biocontrol for cabbage was not possible. The biocontrol effect of BB was not found to be significant on leaves of rape. However, a significant effect was found in leaves of broccoli, where the EBRindex was reduced compared to broccoli plants inoculated only with Xcc. No internal symptoms were observed in broccoli and rape in experiment 3. In the sandy loam soil and during the full rainy season (November 1998–February 1999), the biocontrol achieved by BB was seen by the reduction of the external black rot indices on cabbage, rape and broccoli (experiment 4). Also, no significant difference was recorded in the IBRindex using the different treatments and *Brassica* species.

In experiment 5 that was conducted from April to July 1999, the treatment of *Brassica* roots with BB reduced the size of lesions caused by Xcc on the leaves (EBRindex) in cauliflower, cabbage and broccoli (Table 1). Although, cauliflower treated with BB showed a lower IBRindex compared to the control, the difference was not significant. In contrast, the treatment of cabbage with BB reduced the internal black rot symptoms compared to those only treated with the pathogen. Internal black rot symptoms in broccoli were rarely found and no significant differences were registered.

The biocontrol effect of BB on black rot caused by three different strains of Xcc was tested in experiment 6. Plants first inoculated with BB and then with Xcc strains B-147 and 33908 showed lower external and internal black rot indices. No symptom reduction was obtained when black rot was caused by Xcc strain 33907 (Table 1).

Population studies

In experiment 2, using drop plating and colony blotting, BB could be detected in roots of cabbage inoculated with BB until harvest (Figure 1). Bacterial density in plants inoculated with BB differed significantly from that of control samples during the whole experimental period. In control plants, the amount of background bacteria, which reacted positively with our antibody, was very low. Bacterial density measured in stem samples from inoculated cabbages was insignificant and not different from control samples (data not shown).

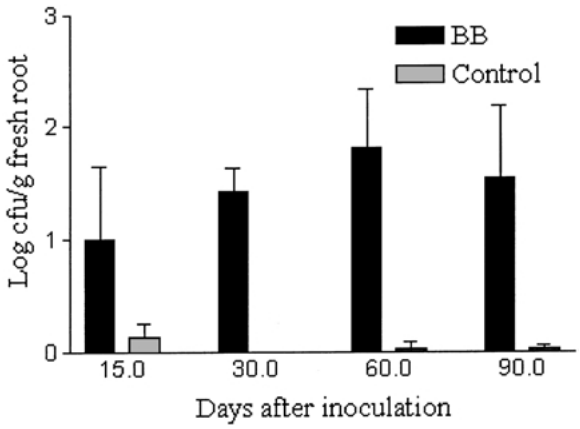

Figure 1. Density of the strain BB in roots of cabbage in field experiment 2. Error bars represent standard deviations.

Discussion

The biological control of black rot using the strain BB (*B. subtilis*) has been proved to be possible on different *Brassica* species during both the dry and short rainy seasons in Zimbabwe. In these seasons, biocontrol was obtained in three consecutive growing seasons with cabbage and in one growing season with three different *Brassica* species. In the first growing season (experiment 1), black rot control was showed by the reduction of the number and size of lesions on the external cabbage leaves and in the absence of internal black rot symptoms. In the second growing period (experiment 2), biological control of black rot was also observed in plants treated with BB as shown by the reduction of the external and internal symptoms. Despite a short rainy period and occurrence of internal symptoms of black rot, the external black rot indexes were generally similar compared to experiment 1, where no rain had fallen during the growing period.

Biological control of Xcc has been previously reported by Mguni (1996) and Assis et al. (1998). Besides reporting biocontrol under greenhouse and growth chamber conditions, Mguni (1996) has also showed reduction of black rot incidence up to 90.3% in cabbages treated with *Bacillus* spp., including the strain BB, in a 90-day field experiment conducted in Zimbabwe. Similarly, Assis et al. (1998) reported

biological control of black rot on cabbage 18 days after inoculation with the pathogen when using an endophytic strain of *Kluyvera ascorbata* under field conditions in an experiment conducted in Brazil.

The application of the antagonist BB on the roots led to the reduction of black rot symptoms on stem and leaves. Interestingly in experiment 2, BB was found to persist endophytically in roots of cabbages throughout the whole growing season, but it was hardly detected within the stem. According to Kloepper et al. (1999), induced systemic resistance might be one of the most important operating mechanisms when dealing with biocontrol of systemic plant pathogens. Many other studies have reported induced systemic resistance triggered by bacterial inoculation (Van Peer et al., 1991; Benhamou et al., 1996; Wilhelm et al., 1998). Although endophytes are able to penetrate roots via natural wounds that appear at the sites of emergence of secondary roots (Agarwal and Shende, 1987; Wulff, 2000), the root tips were removed to facilitate the penetration of endophytes into the plant in experiments 1 and 2. However, data on the ability of BB to penetrate roots via natural wounds were obtained in other greenhouse experiments conducted with seed and root inoculated *Brassicas* (Mguni, 1996; Wulff, 2000). In experiment 2, BB was detected in roots of plants throughout the growing season. The BB population within cabbage roots did not seem to vary much, it fluctuated between log 1 and log 2 CFU per g fresh root which is low. Similarly, Jacobs et al. (1985) observed minimal changes in the population of endophytic bacteria in sugar beet during the growing season. However, BB seems not to be able to move and establish in the upper plant parts, since no significant differences were obtained between stem samples of control and inoculated plants.

During the full rainy season, inoculation of broccoli with BB resulted in biological control in both the clay and sandy loam soil (experiments 3 and 4). No control was obtained in the clay loam soil on cabbage and rape. The biocontrol effect in the latter two *Brassica* species seems to be ineffective when optimal conditions for the spread of the pathogen, such as rainfall and high temperature, are present. Similar observations were made by Bell et al. (1995), when they used endophytic bacterial strains to control crown gall disease in grapevine. The antagonistic activity was very strong *in vitro* and in soil, but *in planta* inhibition was only noticed when crown gall severity in plants was low to medium (<47%) (Bell et al., 1995). In our experiment, amount of rainfall and soil type seem to affect the biocontrol activity of BB against black rot, since no biocontrol was observed during the full rainy season in the clay loam soil. In the rainy season, the constant spreading of Xcc by water splashes make the control of Xcc very difficult. In the sandy loam soil during the full rainy season, limited biological control effect was observed in cabbage and rape. In this soil, surface rainwater quickly percolated in deeper layers of the soil, and thus the humidity around plants might be lower compared to the clay loam soil.

Besides environmental conditions, the species of the host-plant seems to affect the biocontrol activity exercised by BB. Broccoli was the only tested species, which seemed to be protected against black rot by BB both in the dry and rainy seasons and in the clay loam and sandy loam soils. This observation is in agreement with an experiment conducted by Mguni (1996), who has showed that broccoli was among the less susceptible *Brassica* when infested with a combination of three Xccs. Whereas cabbage and cauliflower were the most susceptible followed by rape. In brassicas, altered stomatal structures, fewer hydathodes on the leaves and lower ability to form guttation drops seem to be important factors making the host more resistant to Xcc (Dane and Shaw, 1996). Thus, in the rainy season, farmers should go for a strategy where combination of more resistant varieties, biocontrol and cultivation in lighter soils would contribute to reduce black rot severity.

In experiment 5, conducted in the dry season, BB was again efficient in controlling black rot in cabbage, rape and broccoli. The results obtained for cabbage (EBRindex) in this growing season are similar to the ones obtained in experiment 1. In experiment 1, root tips were removed while in experiment 5 it was not. In the previous experiments under greenhouse conditions, BB was also found to colonise brassicas endophytically after seed treatment and could be detected using direct platting, immunoblotting and transmission electron microscopy (Wulff, 2000). However, in the present study, the presence of BB was only searched and demonstrated in plants from which the root tips were removed (field experiment 2).

Besides controlling black rot caused by the pathogenic Xcc strain B-147, strain BB also controlled black rot of cabbage caused by strain 33908 (experiment 6). Strain B-147 and 33908 might have similar pathogenicities since their EBRindex and IBRindex were similar. However, no biological control effect was obtained when black rot was caused by strain 33907 that like strain 33908 also was isolated from Zimbabwe (Mguni, 1996). The reason might be due to the aggressiveness

of this strain, which caused more severe leaf symptoms compared to the other two strains.

In the present study, it has been shown that the biological control agent BB can be used successfully during the dry and short rainy season against black rot in four different *Brassica* species in Zimbabwe. Under conditions extremely favourable to the pathogen, that is, during the full rainy season and in clay loamy soil, biological control using BB was only obtained for broccoli. Strain BB was shown to colonise endophytically roots of cabbage throughout the growing period in one of the experiments when the root tips were cut. The root dipping technique had the advantage that the inoculum was applied fresh and its application was simple, and since Zimbabwean farmers use transplantation for cultivating brassicas, the technique could be easily adapted to the local conditions. Despite the obtained results, additional studies are still needed to improve biocontrol with the *Bacillus* strain BB before recommending it to subsistence farmers.

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