CHAPTER 5

EFFECT OF CALCIUM SOIL AMENDMENTS IN INCREASING RESISTANCE OF POTATO TO SOFT ROT PATHOGENS

Abstract

Dickeya and *Pectobacterium* species cause blackleg / soft rot disease complex on potato in Zimbabwe. The disease is seedborne and difficult to control. This research focuses on ways of increasing the inherent resistance of potato plants and tubers to blackleg and soft rot. The effect of calcium soil amendment on phenolic compound formation in potato peels and ensuing tuber resistance to the pathogens was investigated. Two field experiments were conducted at the University of Zimbabwe campus plots in 2008 and 2009 summer seasons. Sprouted tubers of cv. BP1 were inoculated with a mixture of *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliensis* and *Dickeya dadantii*. The inoculated tubers were planted in plots treated with different fertilizer combinations. The treatments were: 1) compound S (7N: 21P: 8K) + ammonium nitrate 2) compound D (7N: 14P: 7K) + calcium nitrate 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. Blackleg incidence and severity were significantly lower ($P < 0.05$) in the plots where calcium nitrate was applied in both experiments. Soft rot incidence in the progeny tubers was also significantly reduced by the calcium treatment. In addition, calcium significantly reduced ($P < 0.05$) soft rot losses of tubers in storage. Total soluble phenols and calcium content were measured in leaves and tuber peels at physiological maturity. Phenolic compounds were identified as chlorogenic, caffeic and ferulic acid using HPLC analysis. Chlorogenic acid and calcium content were significantly higher ($P < 0.05$) in plants grown in calcium treated plots. Calcium reduced maceration effect of the bacteria, significantly smaller ($P < 0.05$) rotting zone diameters were recorded on tubers harvested from the calcium treated plots. This study shows that soil amendments of calcium reduce blackleg and soft rot diseases in potato and increases concentration of calcium and chlorogenic acid in tuber peels.

5.1 INTRODUCTION

Bacterial soft rots caused by *Pectobacterium atrosepticum* (*Pa*) (Van Hall) Dye and *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) (Jones) Bergy *et al.* are a major cause of potato tuber loss in the field, storage and transit in many parts of the world (Pérombelon and Kelman, 1980). Potato blackleg is a seedborne disease caused mainly by *Pectobacterium atrosepticum* under cool environments. *Pectobacterium carotovorum* subsp. *brasiliensis* (*Pcb*), *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya* spp. can also cause blackleg and soft rot diseases under different environmental conditions.

In Zimbabwe, potato growers face the challenge of significant post harvest losses of potato tubers (20 – 60%) due to soft rot (Manzira, 2010). Severe blackleg / soft rot disease complex outbreaks occurred in the 2008 / 9 growing season, causing huge economic losses. The causal agents were *Pcb*, *Pcc*, *Pa* and *Dickeya dadantii* (Chapter 3, this study). The pathogens macerate plant tissues by the production of large quantities of pectolytic enzymes (Flego *et al.*, 1997). *Pectobacterium* spp. secrete pectinases that induce polyphenoloxidase activity in the hosts, which subsequently oxidises phenols, forming a black margin around the infection site. The margin restricts pathogen from spreading (Lovrekovich *et al.*, 1967) Most of the potato cultivars grown in Zimbabwe have some level of susceptibility to soft pathogens (Ngadze *et al.*, 2012 in press).

Seed tubers almost always carry latent infections of soft rot pectobacteria in the lenticels and wounds (Pérombelon, 2002) where they multiply aggressively under favourable environmental conditions until they reach a critical population density of between 10^7 and 10^8 cfu ml^{-1}. They colonize and multiply in the apoplast before plant cell death. A number of factors trigger *Pectobacterium* and *Dickeya* spp. to produce pectolytic enzymes when the tubers are in storage. These factors can be changes in water potential (Kelman *et al.*, 1978),

membrane permeability, intracellular concentrations of reducing sugars (Otazu and Secor. 1981), polyphenol oxidase (Lovrekovich *et al.*, 1967; Tripathi and Verma, 1975) or the phytoalexin rishitin (Lyon *et al.*, 1975). The concentration of oxygen within the tuber tissue is of paramount importance and can decrease rapidly if a film of water is maintained on the tuber surface for several hours (Burton and Wigginton, 1970). At low oxygen conditions the tuber becomes more susceptible to attack by even small numbers of bacteria (De Boer and Kelman, 1978). The best management strategy of blackleg and soft rot disease of potatoes is to suppress or prevent multiplication of soft rot bacteria (Pérombelon, 2002).

Calcium is an essential nutrient and considered one of the most important nutrients associated with plant defense (Datnoff *et al.*, 2007). It has been implicated in the interaction between plant pathogenic bacteria and their host plants. Calcium confers some resistance to pests and diseases in plants via its influence on growth pattern, anatomy, morphology and chemical composition of the plant. Increased plant calcium has been shown to enhance resistance to plant tissue macerating bacterial phytopathogens (Perombelon and Kelman, 1980; McGuire and Kelman, 1984; 1986). Calcium is involved in eliciting signal transduction pathways and in membrane and cell wall integrity (McGuire and Kelman, 1984; Busse and Palta, 2006; Datnoff *et al.*, 2007). Calcium increases the resistance of potato stems and tubers to maceration by pectolytic enzymes such as pectate lyase and polygalacturonase (Flego *et al.*, 1997). Bain *et al.* (1996) reported a significant reduction in blackleg incidence during the growing season after preplant application of gypsum ($CaSO_4$), although the effect decreased towards the end of the season. It also enhances the structural integrity of cell walls and membranes. Adjustments in mineral nutrition could reduce disease severity (Mashner, 1995; Datnoff *et al.*, 2007).

Some nutrients, especially silicon stimulate host defenses in plants by stimulating the production of phenolic compounds and phytoalexins in response to pathogen infection. This

can enhance the activity of defense-associated enzymes (Hammerschmidt, 2005). Infected plants have increased polyphenol oxidase activity and phenol concentration. (Lovrekovich *et al.,* 1967). Phenols and phytoalexins play an important role in defense mechanisms to soft rot bacteria (Ghanekar *et al.*,1984). Potato tubers contain relatively low levels of phenolic compounds (Ramamurky *et al.,* 1992). Wegener and Jansen (2007) reported that the phenolics and anthocyanins were responsible for resistance expression in tuber tissues. Ganekar *et al.,* 1984 found that chlorogenic, caffeic and ferulic acid possess antibacterial activity against soft rot pathogens. The total soluble content was directly proportional to the inhibition of soft rot bacteria (Kumar *et al.,* 1991).

Although calcium has been reported to be effective in disease management in different crops (Benson, 2009), including blackleg and soft rot in potato (McGuire and Kelman, 1984; 1986), the effect of this nutrient on disease resistance in potatoes has not been investigated in Zimbabwe. In addition and most Zimbabwean potato farmers do not apply calcium fertilizers to crops. Some of the soils in Zimbabwe have acidic low CEC and base saturation, possibly leading to calcium and / or magnesium deficiencies in the potato growing regions where growers are do not commonly apply these nutrients. The objective of this study was to determine the effect of calcium on resistance of potato to blackleg and soft rot pathogens and to measure the concentration of phenolic compounds in plants grown in calcium amended soils.

5.2 MATERIALS AND METHODS

5.2.1 Experimental site

Two experiments were conducted at the University of Zimbabwe (UZ) campus in the 2008 (experiment 1) and 2009 (experiment 2) summer seasons (August - December). The UZ campus is situated in Harare (17º50′ South and 31º30′ East) at an altitude of 1500m above

sea level. The area is characterized by fersiallitic red clay soils with more than 40% clay and receives an annual rainfall of between 800 to 1000mm. Average temperatures during the growing season ranged from 20 to 25ºC. The fields were planted to Brassicae prior to the experiment. The crop was hoe weeded when necessary and pests were controlled with carbaryl used at the recommended rate.

5.2.2 Experimental design

The experiment was laid out as a randomized complete block design with four treatments. The treatments were: 1) compound S [7N: 21P: 8K] + ammonium nitrate; 2) compound D [7N: 14P: 7K] + calcium nitrate (19Ca : 15.5N); 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. The different fertilizer treatments were applied to the appropriate plots. Three blocks were used in the experiment and the treatments were replicated three times in each block. Certified potato seed of cultivar BP1 was used in the experiment.

5.2.3 Agronomic practices

Compounds D (7N: 14P: 7K) and S (7N: 21P: 8K) fertilizers were applied as basal fertilizers at a rate of 1000 kg ha^{-1} in the relevant treatments. Calcium nitrate at a rate of 250 kg ha^{-1} was mixed with basal fertilizer for the relevant treatments. The first application was applied at the planting stations after opening the furrows, and then slightly covered with the soil before planting the tubers at a depth of 10cm. Ammonium nitrate and the second calcium nitrate application were applied as top dressing at a rate of 250 kg ha^{-1} and 250 kg ha^{-1}, respectively, 6 weeks after crop emergence. The fertilizer was placed about 5 cm away from the plants to avoid scorching. The fields were irrigated when necessary and 300 mm of water was applied to the field as a supplement for the whole season.

5.2.4 Chemical properties of soil

Samples were obtained from the two field trial sites on 11 August 2008 and on 10 August 2009, respectively, prior to planting the potatoes. Soil was sampled from 5 random locations (20 cm deep) in each field. Soil chemistry analysis was conducted at the Soil and Plant Analysis Laboratory, University of Zimbabwe using a method described by Baysal *et al.* (2008) with minor modification. The soil samples were dried at room temperature for a week, ground and passed through a 2 mm sieve. Soil pH was analyzed in a 1:25 (wt:wt) water suspension with a pH meter (model Hanna 2210, Sigma-Aldrich). pH was measured before the application of the various fertilizers only. Cation exchange capacity (CEC) and exchangeable cations (CaO, MgO, K_2O) were measured by the shaking extraction method (Page *et al.*, 1982).

5.2.5 Bacterial cultures

Bacterial strains of *P. atrosepticum* (LMG 2386^T, Belgian Coordinated Collections of Microorganisms), *P. c.* subsp. *brasiliensis* (ATCC BAA-419 *Pcb* Strain 371, American Type Culture Collection) and *D. dadantii* (*Erwinia chrysanthemi* 3937, Scottish Crop Research Institute) were used in the study. They were grown for 24 hours at 25˚C in a shaken culture of 25 ml Luria Bertani (LB) broth (pH 7.0) supplemented with 0.1 % pectin from citrus fruits (Sigma) and 0.1 % lyophilized potato cell sap. After centrifugation at 5000 rpm for 5 minutes (4˚C) the bacteria were washed with sterile water, centrifuged again and re-suspended in sterile water before adjusting to the appropriate density (OD_{600} – 0.1).

5.2.6 Inoculation method

The seed tubers were sprouted and inoculated with a mixture of the three pathogens using the method described by Hélias *et al.* (2000) with minor modifications. Three pathogens were used in the study as opposed to the one pathogen used by Helias *et al.* (2000). The sprouted

seed potato tubers were dipped in the inoculum for 15 minutes and dried overnight before planting.

5.3 MEASUREMENTS

5.3.1 Disease incidence

Diseased plants were defined as those showing at least one stem with blackleg or blackleg associated symptoms (soft rot, wilting, internal and external darkening on stems), excluding non-emergence. The plants showing symptoms were then expressed as the percentage of the total number of plants that emerged (Hélias *et al.*, 2000) per treatment per block.

5.3.2 Disease severity

Disease severity assessment was based on a scale developed by Hélias *et al.* (2000) and was carried out fortnightly per plant until the plants reached physiological maturity: The scale was defined as:

i) Wilting / chlorosis (Wlg / chl),

ii) Blackleg (Bl)

iii) Haulm desiccation - corresponding to death of the stems of the plant

iv) Plant death - (Dth) – complete desiccation of all stems

v) Symptomless plants, recorded as healthy (Hth).

5.3.3 Plant mineral analysis

The mineral content of the various tissues of the potato plant was determined by inductively coupled plasma-optical emission spectrometry by the Soil and Plant Analysis Laboratory, University of Zimbabwe. For determination of calcium in the leaf tissue, the top fully expanded leaf was collected from each of 10 plants / block at the flowering stage and these were bulked. For tuber analysis, five tubers were randomly selected at harvest from each treatment

per block and taken as the representative sample. Calcium content in tubers was determined using the method described by McGuire and Kelman (1984) with minor modifications; only the peel was used.

5.3.4 Extraction of phenolic compounds

Five tubers randomly selected from harvested tubers of each treatment were washed under running water and peeled with a potato peeler. Tuber peels were freeze-dried for five days and then ground to a fine powder. Two hundred milligrams of fine powder was passed through a 1mm sieve and placed in a 1.5 ml micro centrifuge tube for extractions. Aliquots of 1 ml of a cold mixture of methanol: acetone: ultra-pure water (7:7:1, v:v:v) were added, vortexed and ultrasonified for 5 min. After sonification, samples were shaken for 20 min at 160 rpm while on ice. Samples were centrifuged for 5 min and the supernatant of each sample was transferred to a 20 ml centrifuge tube. This process was repeated in triplicate and supernatants finally evaporated in a laminar flow cabinet at room temperature. The residue was dissolved in 1ml sterile, ultra-pure water. Finally, samples were filtered through 0.45 µm, 25 mm, Ascrodise, GHP, syringe filters (Separations, South Africa). Samples were stored at 4 ºC until analysis using reverse phase – high performance liquid chromatography (RP – HPLC).

5.3.5 Reverse Phase – High Performance Liquid Chromatography

For identification and quantitative analysis of samples, 10 µl of purified extract per sample was analyzed using RP - HPLC (Hewlett Packard Agilent 1100 series) with a UV diode array detector, at 325 and 340 nm. Separation was achieved on a Gemini 3µ, C18, 110A (Phenomenex®) reverse phase column (250 mm length, 5 µm particle size, 4.6 mm inner diameter). A gradient elution was performed with water (pH 2.6 adjusted with H_3PO_4) and acetonitrile (ACN) and consisted of 0 min, 7% ACN; 0 – 20 min, 20% ACN; 20 – 28 min, 23%

ACN; 28 – 40 min, 27% ACN; 40 – 45 min, 29% ACN; 45 – 47 min, 33% ACN; 47 – 50 min, 80% ACN. Solvent flow rate was 0.7 ml min^{-1}. Identification of the phenolic compounds was done by comparing their retention times and UV apex spectrum to those of standards: chlorogenic acid, caffeic acid and ferulic acid. The column was re-equilibrated with initial conditions for 10 min, after each run.

5.3.6 Yield assessment and yield losses

At harvest tubers from each plant were examined for soft rot disease. Healthy tubers were collected in sacks. The weight of tubers was recorded at harvest using a digital weighing scale (model DS410, Teraoka South Africa). Rotten progeny tubers were also weighed and mass converted to percentage yield loss. The mother tubers were inspected for rotting and number of rotten seed tubers was also expressed as a percentage of number of tubers planted per treatment.

5.3.7 Losses of tubers in storage

Forty progeny tubers were randomly selected from each treatment. The tubers from the various treatments were placed in 10 kg pockets of meshed black polythene material, and then stored at 25 ˚C and 60 % relative humidity. The potato tubers were stored for eight weeks and assessed fortnightly for rotting. The number of rotten tubers in each pocket was noted and converted to a percentage.

5.3.8 Maceration of potato tuber tissue by bacteria

Eight Randomly selected seed tubers from each treatment were washed thoroughly under running tap water and surface-disinfected by dipping in 70% ethanol for 5 min, before being cut longitudinally into halves (giving 16 halves). Each tuber half was inoculated with 10 mm diameter filter paper discs (Whatman's No. 1), that had been soaked for 10 min in a bacterial

suspension of *Pcb* (only Pcb was for this test). For the control, filter paper discs were soaked in sterile distilled water for 10 min and placed on tuber halves. One filter paper disc was placed in the pith and four around the edges of each tuber half. The experimental design was a Randomized Complete Block Design (RCBD) with four replicates per treatment (4 half tubers per treatment). After inoculation and incubation at 25˚C for 24 h the filter paper discs were removed and the diameter of each rotting zone was measured. The tuber halves were incubated for a further 24 h and the rotting zone diameter was measured again.

5.3.9 Statistical analysis

The disease progress curves were constructed from severity scores and area under the disease progress curve (AUDPC) was calculated for each treatment using trapezoidal integration (Sigma Plot, 2000).

The trapezoidal integration formula used is:

Trapezoidal integration = yi[(xi=1)-xi] + (1/2)[(yi+1)-yi][xi+1)-xi]

Where xi = time of scoring

Yi = severity score

All disease severity data were square root (x +0.5) transformed before analysis. Analysis of variance (ANOVA) was carried out using a Minitab Version 12 (2001) of Statistical Package. Disease incidence was analysed using repeat measures analysis.The standard error of difference (SED) calculated was used for mean separation when $P<0.05$. The rest of the data was subjected to analysis of variance (ANOVA) using the GenStat statistical package (GenStat, 2002 Release 6.1, Lawes Agricultural Trust, Rothamstead) (Payne, 2003). Means were separated using Fisher's protected Least Significant Difference (LSD at $P < 0.05$).

5.4 RESULTS

5.4.1 Blackleg incidence

Calcium nitrate significantly reduced ($P < 0.05$) blackleg incidence by more than 20% in experiments 1 and 2 at 14 Weeks After Crop Emergence (WACE). The lowest disease incidence was recorded from 2 – 14 WACE in calcium treated plots and the highest disease incidence of 50% was recorded in the plots treated with compound D + ammonium nitrate in both experiments (Figures 1A and B). In experiment 2 no disease was observed in calcium treated plots at 2 WACE (Figure 1B). There was no significant difference in disease incidence in plants grown in plots treated with compound D or compound S top dressed with calcium nitrate in experiments 1 and 2 (Figures 1A and B).

5.4.2 Blackleg severity

Blackleg severity was significantly higher ($P < 0.05$) from 2 to 12 WACE in the plots top dressed with ammonium nitrate in both experiments 1 and 2. The highest disease severity score of more than 6.5 was recorded in the plots treated with compound D + ammonium nitrate in both experiments 1 and 2. This was not significantly different from the disease score recorded for compound D + ammonium nitrate in experiment 2 (Figures 2A and B). Calcium nitrate in combination with either compound D or S significantly reduced ($P < 0.05$) blackleg severity in both experiments. No disease symptom was observed in calcium treated plots in either experiment at 2 WACE.

5.4.3 Soft rot incidence

There were no significant differences in the percentage of rotten mother tubers between treatments (Table 3). The lowest percentage of rotten progeny tubers was recorded in plots treated with calcium nitrate. There was no significant difference in the percentage of rotten progeny tubers between compound D + calcium nitrate and compound S + calcium nitrate in

both experiments 1 and 2. The highest percentage of rotten progeny tubers was recorded in plots treated with compound D + ammonium nitrate in both experiments (Table 3).

5.4.4 Soil and plant nutrients

In 2008 and 2009 preplant soil values were 6.0, 6.7 pH; 193.4, 207.8 ppm CaO; 32.4, 45.8 ppm Mg and 24.3, 29.4 ppm K respectively. CEC in these soils ranged from 14.9 to 15.6 Me/100g. The concentration of calcium in both the leaves and tuber peels was significantly higher ($P < 0.001$) in plants grown in the plots amended with calcium nitrate. The amount of calcium in the leaves was almost double that which was recorded for the tubers in both experiment 1 and 2 (Table 3).

5.4.5 Phenolic compounds

Ferulic acid was not detected at the set parameters in all samples and the results are therefore not included. Tuber peels from all treatments showed low levels of caffeic acid and there were no significant differences between the treatments. However chlorogenic acid concentration was significantly higher ($P < 0.05$) in tuber peels from the calcium treatments. Significantly higher ($P < 0.05$) concentrations of chlorogenic acid were recorded in plots where $Ca(NO_3)_2$ was applied in combination with either compound D or S for both experiments 1 and 2 (Table 2).

5.4.6 Yield assessment and yield losses

The highest yields of 33.87 ton ha^{-1} and 39.27 ton ha^{-1} were recorded in the plots treated with compounds S + ammonium nitrate, for experiments 1 and 2 respectively, while the lowest yields were for the compound D + $Ca(NO_3)_2$ treatments. Significantly higher ($P < 0.05$) yield losses (progeny tubers rotting before harvesting) of 16.53% and 39.27% were recorded in 2008 and 2009 in the plots treated with compound D + ammonium nitrate than in other plots.

5.4.7 Losses in storage

The highest number of rotten tubers was recorded at 4, 6 and 8 weeks after harvesting for the ammonium nitrate top dressing treatments. Calcium significantly ($P < 0.05$) reduced losses in storage. At 2 weeks after harvesting, the percentage of tubers which rotted in storage ranged from 0 % recorded for tubers harvested from plots treated with compound S + calcium nitrate to 5 % recorded for plots treated with compound D + ammonium nitrate for both experiments 1 and 2 (Figures 5 A and B). At 6 weeks, the highest number of rotting tubers was recorded in harvested from plots treated with compound D + ammonium nitrate in experiment 1 and for both compound D + ammonium nitrate and compound S + ammonium nitrate for experiment 2. At 8 weeks tubers from plots top dressed with ammonium nitrate recorded a significantly higher percentage of rotting, more than 20 % (Figures 5 A and B). Calcium significantly reduced the number of tubers which rotted in storage for both experiments 1 and 2, but the total percentage of rotten tubers was lower in experiment 1 than in experiment 2.

5.4.8 Maceration of potato tuber tissue by bacteria

Addition of calcium nitrate to the soil significantly reduced ($P < 0.05$) the maceration effect of *Pectobacterium carotovorum* subsp. *brasiliensis*. Significantly smaller ($P < 0.05$) rotting zone diameters were recorded on tubers harvested from plots treated with $Ca(NO_{3)2}$ in both experiments (Table 5.2).

5.5 DISCUSSION

Calcium is an essential mineral that has been shown to be important in many physiological processes, such as plant defense. A deficiency of calcium in the plant can create conditions favourable for pathogen infection (Rahman and Punja, 2007). Calcium has been implicated in the interaction between plant pathogenic bacteria and their hosts. Increased plant calcium

has been shown to enhance resistance to plant tissue macerating bacterial phytopathogens (Perombelon and Kelman, 1980; McGuire and Kelman, 1984; 1986).

In this study the concentration of calcium was higher in the leaves than in the tubers, which was not surprising because stems and leaves are richer in calcium compounds than underground storage organs. This is related to the inability of phloem to transport calcium from the leaves to the root system (Demarty *et al.*, 1984).

Addition of calcium to the soil resulted in increased levels of calcium in the plant. The plants with higher concentrations of calcium recorded low disease incidence and severity showing that calcium is beneficial in increasing the resistance of potatoes to blackleg disease. This supports the findings of McGuire and Kelman (1984), who reported that more susceptible potato varieties have low calcium levels in the tubers, although the concentration of calcium recorded for the plants in this study were higher than those recorded by McGuire and Kelman (1984). The differences in calcium concentrations might be due to the soil types used, in this study the researchers grew the potatoes in red fersiallitic soils with high CEC whereas McGuire and Kelman used sandy soils with low CEC. Furthermore different calcium formulations were used in the two studies. McGuire and Kelman (1984) used $CaSO_4$ as a source of calcium and in this study the source of calcium was calcium nitrate. Environmental conditions might have also contributed to the differences in calcium concentrations recorded in plant tissues of the two studies. Residual calcium recorded in the soil before planting the two experiments might have contributed to the high concentrations of calcium recorded in all treatments, including those planted in plots not treated with calcium.

Low blackleg and soft rot incidences could also be attributed to resistance associated with a high calcium concentration in the cell walls due to the application of calcium nitrate as a basal fertilizer. This is supported by the findings of Bain *et al.* (1996) and Flego *et al.* (1997) who

reported that pre-planting application of calcium as calcium silicate or gypsum ($CaSO_4$) increased calcium concentration in the cells making the plants more resistant to blackleg development. Calcium also increases cell wall integrity, promotes thicker skin netting and ensures proper cell signaling of pathways involving calmodulin; thus reducing disease incidence and severity (Rahman and Punja, 2007). Calmodulin, a Ca^{2+} binding protein, activates and regulates a number of key enzymes, regulates Ca^{2+} transport within the cell and mediates transfer thereof to the vacuoles. Ca^{2+} is also regarded as an important secondary messenger in the elicitation of phytoalexins, which also play an important role in host defense mechanisms (Zook *et al.*, 1987). There was no correlation between soft rot incidence and blackleg incidence.

A high calcium content in host tissues has been correlated with increased resistance to several diseases (Bateman and Lumsden, 1965; Bateman and Millar, 1966; Forster and Echandi, 1975; McGuire and Kelman, 1983; 1984). Plants grown in nutrient conditions of high Ca^{2+} content showed resistance to soft rot diseases. Calcium nitrate significantly reduced the rotting zone diameters in inoculated tuber halves. The increased resistance in tissues with high levels of calcium has been attributed to decreased maceration owing to calcium deposition in the cell wall pectate and structural enhancement of cell wall integrity (McGuire and Kelman, 1984; 1986; Carpita and Gibeaut, 1993). Bateman and Lumsden (1965) suggested that the excess calcium combines with pectin to form calcium pectate, which is resistant to the action of polygalacturonase (PG).

The ability of *Pc.* subsp. *carotovorum* and other soft rot species to macerate plant tissue is dependent on the massive production and secretion of plant cell wall-degrading enzymes, especially pectinolytic enzymes such as polygalacturonase (Peh), pectin lyase (Pnl) and isoforms of pectate lyase (Pel) (Collmer and Keen, 1986). These enzymes are crucial for the

virulence of *Pectobacterium* subsp. and mutations which affect production and secretion of these enzymes lead to reduced virulence (Reeves *et al*., 1994; Pirhonen *et al*., 1991; 1993). The reduced rotting zone diameters and reduced postharvest losses noted in this experiment could also be attributed to calcium interfering with the production of endopolygalacturonase, an enzyme required in the early stages of infection, which could also in turn lead to reduced virulence. Increased extracellular calcium inactivates the gene which codes for endopolygalacturonase production but does not affect the production of other cell wall degrading enzymes (Flego *et al*., 1997). Inactivation of a single gene encoding a particular pectic enzyme can drastically reduce virulence (Saarilahti *et al*., 1992). Reduced maceration recorded in tubers harvested from the calcium treated plots could have been due to high concentrations of chlorogenic acid and caffeic acid in these tubers. Chlorogenic acid is formed as a defense in potatoes in response to infection or injury (Ghanekar *et al*., 1984). Caffeic acid is known to have antibacterial activity against soft rot bacteria by inhibiting growth (Kumar *et al*., 1991). The combination of caffeic acid and chlorogenic acid may significantly inhibit infection by soft rot pathogens (Ghanekar *et al*., 1984; Kumar *et al*., 1991).

The yields recorded for the plants grown in compound S + ammonium nitrate were in line with the average yields recorded by farmers in Zimbabwe who grow the same variety. The average yield for BP1is estimated to be at 30 t/ha (Manzira, 2010). The lower yield losses recorded in the calcium treated plots could be attributed to the lower nitrogen content in calcium nitrate which was used for top dressing the crop. Ammonium nitrate contains 34 % nitrogen while calcium nitrate contains 15.5 % nitrogen. Lower yields were recorded for plants treated with compound D compared to those grown in plots treated with compound S. These findings support the findings of Manzira (2010), who recommended the use of compound S as a basal fertilizer for potato and compound S also contains a higher content of potash which is required by potatoes.

Chlorogenic, caffeic and ferulic acid are the three main phenolic compounds found in potato tubers and they have antibacterial effects and inhibit growth of soft rot bacteria (Ghanekar *et al.*, 1984; Kumar *et al.*, 1991). Ferulic acid was not detected in all treatments. These results were consistent with the findings of van der Merwe (2009) who did not detect Ferulic acid in tubers grown in silicon amended soil. Although there was no significant difference in the concentration of caffeic acid between treatments, the concentration of caffeic acid was lower than that of chlorogenic acid. This was expected because chlorogenic acid is the storage form of caffeic acid and can be converted to caffeic acid during stress conditions (Ghanekar *et al.*, 1984). Chlorogenic acid was significantly higher in tubers harvested from the calcium amended plots. The results show that calcium has a positive effect on the production of chlorogenic acid which enhances the protection and resistance against soft rot pathogens. Chlorogenic acid is formed in potato tissues in response to infection or injury (Ghanekar *et al.*, 1984). Caffeic acid and chlorogenic acid significantly inhibit soft rot infection when present at the same time (Ghanekar *et al.*, 1984; Kumar *et al.*, 1991).

Several researchers have reported the beneficial effects of calcium in increasing potato resistance against soft rot pathogens (McGuire and Kelman, 1984; 1986; Bain *et al.*, 1996; Flego *et al.*, 1997). This study has confirmed these findings under Zimbabwean conditions. It will be beneficial for the potato growers to supplement calcium in the field in order to reduce the blackleg / soft rot disease complex since calcium improves tuber resistance against the pathogens.

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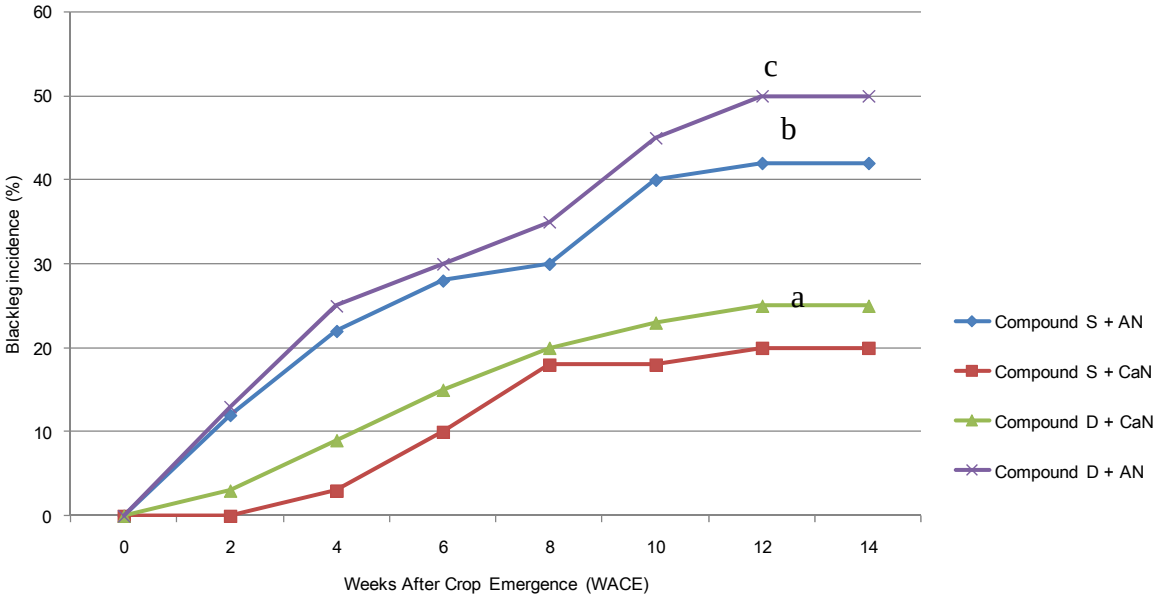
Table 5.1: Chemical composition of soils in the two experimental fields at the UZ campus taken in 2008 and 2009 prior to the experiment.

Year	Field	pH	CEC Me/100g	CaO (ppm)	MgO (ppm)	K_2O (ppm)
2008	Block 4	6.0	15.6	193.4	32.4	24.3
2009	Block 2	6.7	14.9	207.8	45.8	29.4

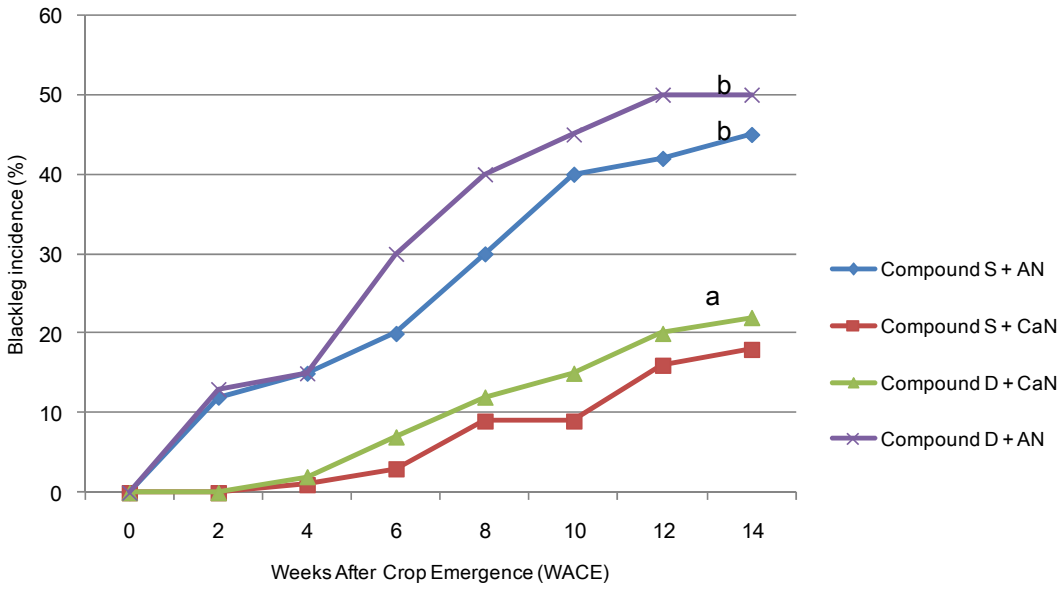
Table 5.2: Soft rot incidence in mother and progeny tubers of BP1 at harvest for experiments 1 (2008) and 2 (2009)

Treatment	Experiment 1 (2008)				Experiment 2 (2009)			
	Yield (tons ha^{-1})	Rotten Mother Tubers (%)	Rotten Progeny Tubers (%)	Rotting zone diameter (mm)	Yield (tons ha^{-1})	Rotten Mother Tubers (%)	Rotten Progeny Tubers (%)	Rotting zone diameter (mm)
CompD+AN	21.52b	41.1	16.53c	29.90b	26.00b	39.7	30.13c	30.00b
CompS+CaN	27.42c	36.1	6.53a	20.10a	32.67c	36.1	3.50a	16.90a
CompD+CaN	16.58a	37.2	5.21a	19.70a	23.73a	35.0	6.44a	18.20a
CompS+AN	33.87d	38.9	9.44b	26.30b	39.27d	38.9	19.55b	28.50b
P-Value	0.003	0.370	<0.001	<0.001	<0.001	0.120	< 0.001	<0.001
SED	1.459	2.830	1.833	0.479	0.827	2.080	3.925	1.174
LSD (0.05)	2.918	NS	3.663	0.954	1.654	NS	7.850	2.348

Means followed by the same letter in a column are not significantly different at LSD 0.05

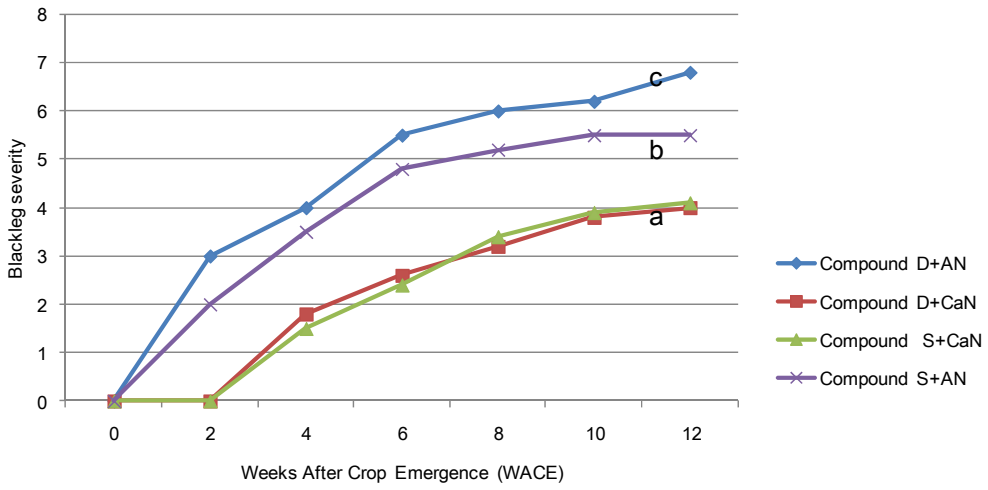


A

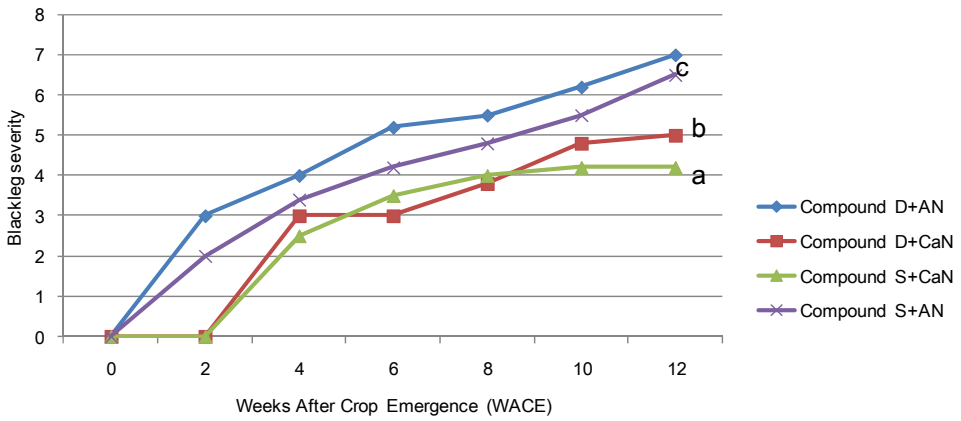


B

Figure 5.1 Field experiments evaluating the effect of calcium application on the incidence of potato blackleg and soft rot in two experiments **A**) 2008 **B**) 2009. Data analysed using repeat measures analysis Different letters denote significant difference according to ANOVA and least significant difference ($P < 0.05$)



A



B

Figure 5.2 Area Under Disease Progress curves calculated for blackleg severity in two experiments **A**) 2008 **B**) 2009. Different letters denote significant difference according to least significant difference ($P < 0.05$).

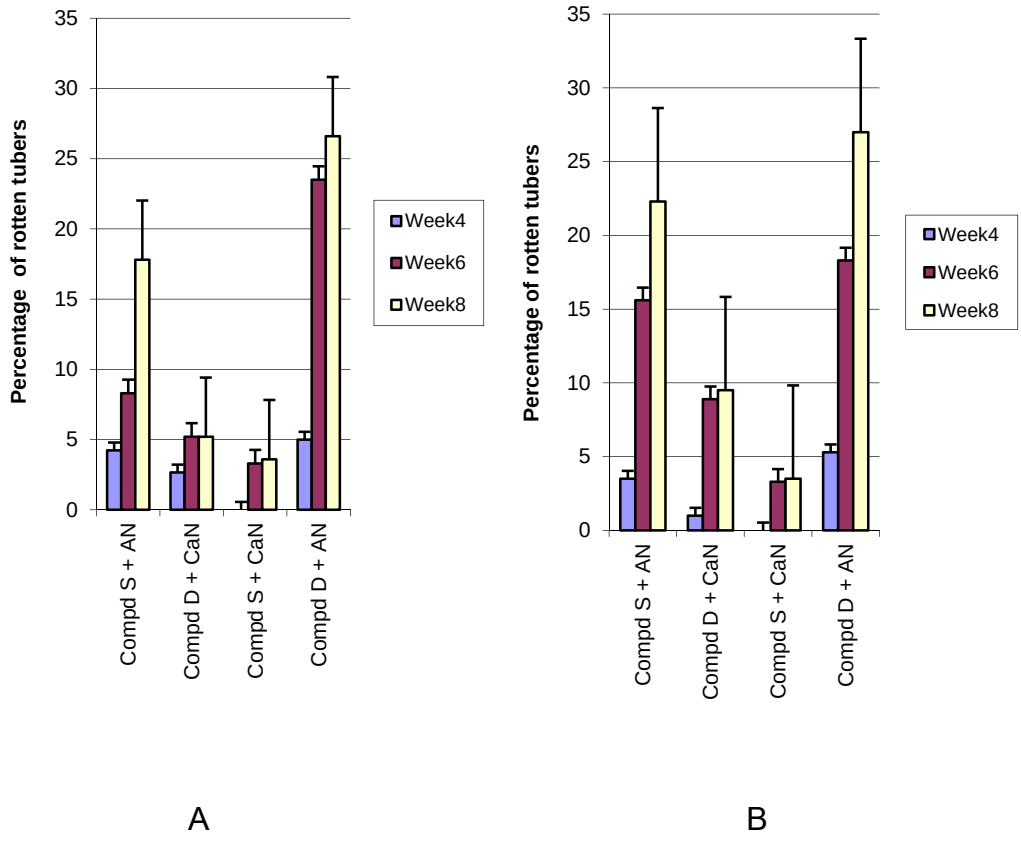


Figure 5.3 Number of tubers which rotted in storage at 4, 6 and 8 weeks after harvesting for experiment 1 (2008) **(A)** experiment 2 (2009) **(B)** ($P < 0.05$). The data represent the mean of three replicates and results were analyzed separately. Error bars on figures represent SED.