

CALCIUM SOIL AMENDMENT INCREASES RESISTANCE OF POTATO TO BLACKLEG AND SOFT ROT PATHOGENS

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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 focused on ways of increasing the inherent resistance of potato plants and tubers to blackleg and soft rot. Two field experiments were conducted at the University of Zimbabwe campus plots in 2008/9 and 2009/10 seasons in order to determine reproducibility of the results. Sprouted tubers of cv. BP1 were inoculated with a mixture of *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliense* 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 (34 %N); 2) compound D (7N: 14P: 7K) + calcium nitrate (19 Ca: 15.5N); 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. Blackleg disease incidence and severity were recorded fortnightly starting from 2 weeks after crop emergence (WACE) while soft rot disease was assessed at physiological maturity. Blackleg incidences and severity were significantly lower ($P < 0.05$) in the plots where calcium nitrate was applied for both experiments. Blackleg disease incidence was reduced by more than 20% in both experiments 1 and 2. Soft rot incidence in the progeny tubers was also significantly reduced by the calcium treatment. In addition, calcium amendment significantly reduced ($P < 0.05$) soft rot losses of tubers in storage. Calcium nitrate was effective in reducing blackleg and soft rot diseases in combination with either compound D or compound S. The yield was significantly higher in plots where compound S was applied as a basal fertilizer than that recorded in plots treated with compound D fertilizer. This study shows that calcium soil amendments reduce blackleg and soft rot diseases under Zimbabwe's growing seasons in red fersiallitic soils. Compound S produces better results in potato production than compound D and farmers should be encouraged to use compound S when growing potatoes.

Key words: potato, disease resistance, blackleg, soft rot, storage losses, calcium



INTRODUCTION

Potato blackleg is a seedborne disease caused mainly by *Pectobacterium atrosepticum* under cool environments at temperatures around 20°C. *Pectobacterium carotovorum* subsp. *brasiliensis* (*Pcb*), *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya* spp. can also cause blackleg and soft rot diseases under different environmental conditions [1, 2]. Blackleg develops at cooler temperatures between 20 to 25°C, while under high temperatures above 30°C the pathogen moves to progeny tubers and causes soft rot [1, 2]. In Zimbabwe, potato growers face the challenge of significant post-harvest losses of potato tubers (20 – 60%) due to soft rot [3]. 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* [2, 4]. Most of the potato cultivars grown in Zimbabwe have some level of susceptibility to soft rot pathogens. Resistant cultivars have not yet been identified [5].

Calcium is an essential nutrient and is considered one of the most important nutrients associated with plant defense [6]. It 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 phytopathogen [7, 8, 9]. Calcium increases the resistance of potato stems and tubers to maceration by pectolytic enzymes such as pectate lyase and polygalacturonase [10]. Bain *et al.* reported a significant reduction in blackleg incidence during the growing season after pre-plant application of gypsum (CaSO₄), although the effect decreased towards the end of the season [11]. Calcium also enhances the structural integrity of cell walls and membranes of the plants. Adjustments in mineral nutrition could reduce disease severity [6, 12].

Although calcium has been reported to be effective in disease management in different crops [13], including blackleg and soft rot in potato [7, 8], the effect of this nutrient on disease resistance in potatoes has not been investigated in Zimbabwe. In addition, most Zimbabwean potato farmers do not apply calcium fertilizers to crops. Some of the soils in Zimbabwe have acidic low Cation Exchange Capacity (CEC) and base saturation, possibly leading to calcium and / or magnesium deficiencies in the potato growing regions where growers do not commonly apply these nutrients. The objective of this study was to determine the effect of calcium in reducing potato blackleg soft rot diseases on potato plants grown under conditions of high temperature and high humidity in red fersiallitic soils.

MATERIALS AND METHODS

Experimental site

Two field experiments were conducted at the University of Zimbabwe (UZ) campus in the 2008/9 (experiment 1) and 2009/10 (experiment 2) summer seasons (August - December). The UZ campus is situated in Harare (17°50' South and 31°30' 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 25°C. The fields



were planted with Brassicae prior to the experiment. The crop was hoe- weeded when necessary and pests were controlled with carbaryl applied at the recommended rate.

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 [34.5 % N]; 2) compound D [7N: 14P: 7K] + calcium nitrate [19Ca: 15.5N]; 3) compound S + calcium nitrate and 4) compound D + ammonium nitrate. Compound S [7N: 21P: 8K] and compound D [7N: 14P: 7K] fertilizers were used in this experiment because compound S is the fertilizer recommended for growing potato in Zimbabwe, but some smallholder farmers opt for compound D, which is cheaper. The combination of fertilizers in these treatments ensured that calcium was present only in treatments 2 and 3. 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.

Agronomic practices

Compounds D (7N: 14P: 7K) and S (7N: 21P: 8K) were applied as basal fertilizers at a rate of 1000 kg ha⁻¹ (recommended rate for growing potatoes in Zimbabwe) in the relevant treatments. Calcium nitrate at a rate of 250 kg ha⁻¹ was mixed with basal fertilizer for treatments 3 and 4. The first application of calcium nitrate was applied after opening the furrows, and then slightly covered with soil before planting the tubers at a depth of 10 cm. Ammonium nitrate and the second calcium nitrate applications were applied as top dressing at a rate of 250 kg ha⁻¹ 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.

Chemical properties of soil

Samples were obtained from the two fields' 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.* [14] 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. Cation exchange capacity (CEC) and exchangeable cations (CaO, MgO, K₂O) were measured by the shaking extraction method [15].

Bacterial cultures

Bacterial strains of *P. atrosepticum* (LMG 2386^T, Belgian Coordinated Collections of Microorganisms), *P. c.* subsp. *brasiliense* (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 at (4°C), the bacteria were washed with sterile water, centrifuged again and re-suspended in sterile water before adjusting to the appropriate density (OD₆₀₀ – 0.1).

Inoculation method

The seed tubers were sprouted and inoculated with a mixture of the three pathogens using the method described by Hélias *et al.* [16] with minor modifications. Three pathogens were used in the study as opposed to the one pathogen used by Hélias *et al.* [16]. The sprouted seed potato tubers were dipped in the inoculum for 15 minutes and dried overnight before planting.

Data Collection

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 [16] per treatment per block.

Disease severity

Disease severity assessment was based on a scale developed by Hélias *et al.* [16] 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).

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.

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 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.

Plant mineral analysis

The mineral content of the various tissues of the potato plant was determined by inductively coupled plasma-optical emission spectrometry at 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 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 & Kelman [7] with minor modifications; only the peel was used.

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 [17].

The trapezoidal integration formula used was:

$$\text{Trapezoidal integration} = y_i[(x_{i+1})-x_i] + (1/2)[(y_{i+1})-y_i][(x_{i+1})-x_i]$$

Where x_i = time of scoring

Y_i = 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) [18]. Means were separated using Fisher's protected Least Significant Difference (LSD at $P < 0.05$).

RESULTS

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 about 50% was recorded in the plots treated with compound D + ammonium nitrate in both experiments (Figs. 1A and B).

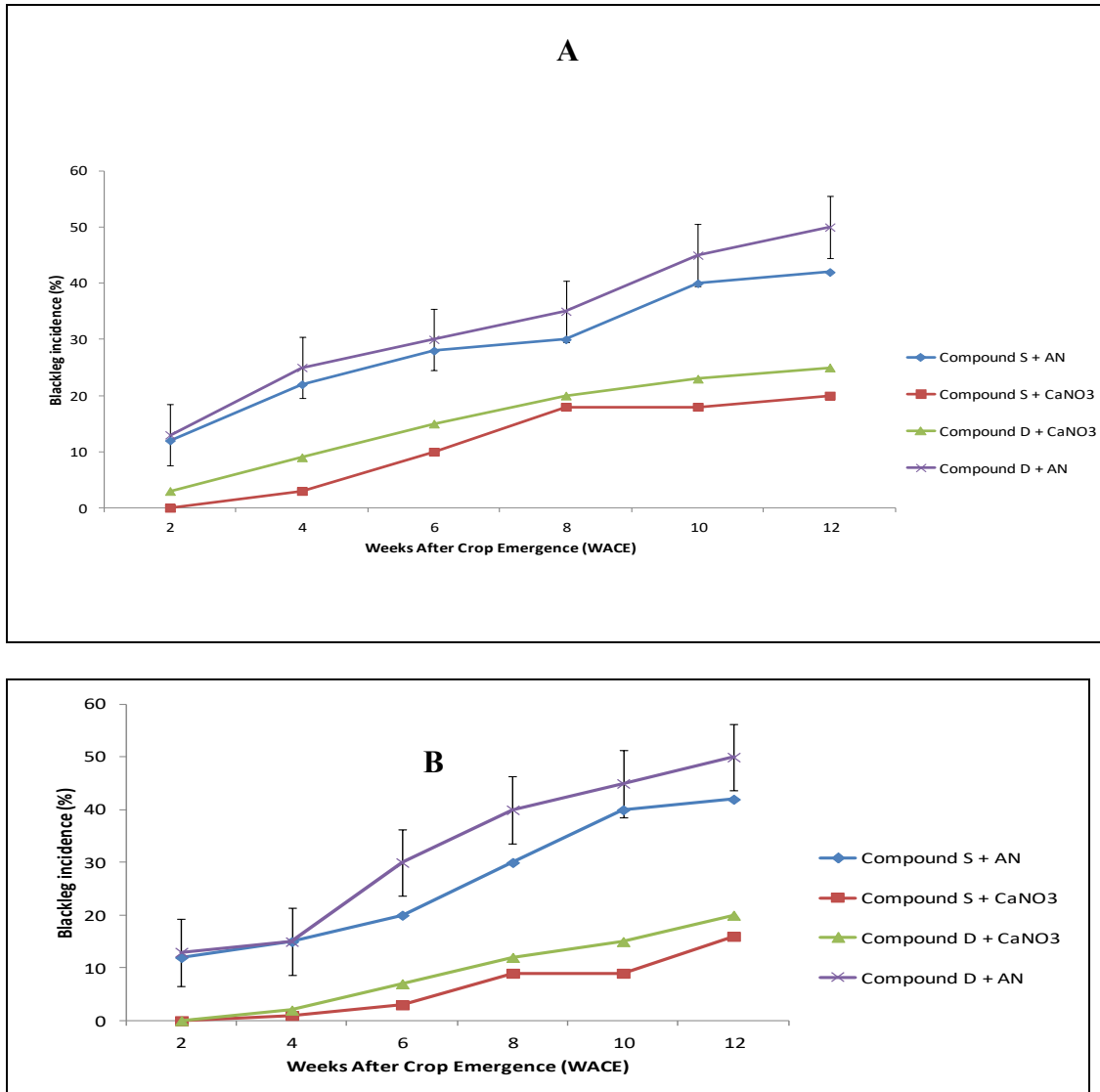


Figure 1: Field experiments evaluating the effect of calcium application on the incidence of potato blackleg and soft rot in two experiments A) experiment 1 2008/9 B) experiment 2 2009/10. Data analyzed using repeat measures analysis

In experiment 2 no disease was observed in calcium treated plots at 2 WACE (Fig. 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 (Figs. 1A and B).

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.

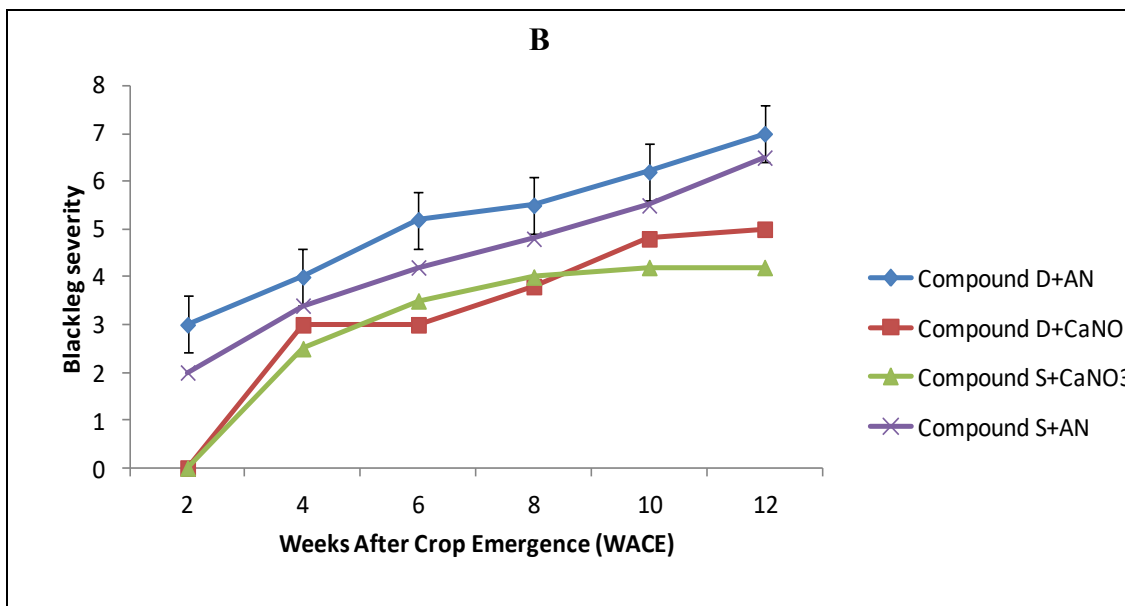
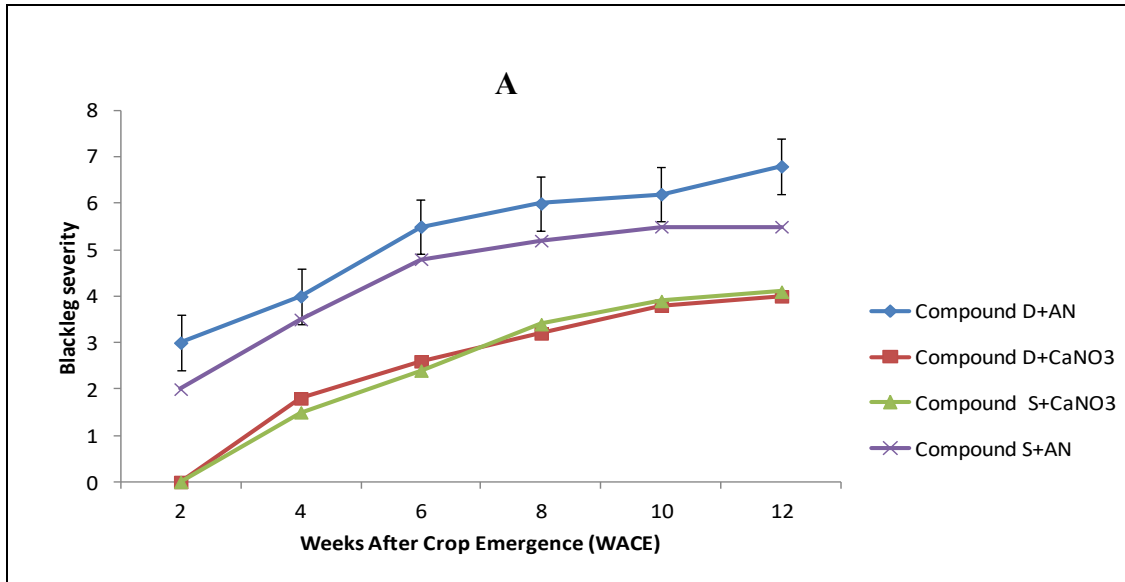


Figure 2: Area Under Disease Progress curves calculated for blackleg severity in two experiments A) experiment 1 2008/9 B) experiment 2 2009/10

Soil nutrients

In 2008 and 2009 pre-plant soil values were 6.0, 6.7 for pH; 402, 126 ppm for CaO; 105, 35 ppm for Mg and 24.3, 29.4 ppm for K, respectively. Calcium Exchange Capacity (CEC) in these soils ranged from 4.9 to 5.6 Me/100g (Table 1).

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(NO₃)₂ treatments (Table 2). Significantly higher ($P < 0.05$) yield losses (progeny tubers rotting before harvesting) of 16.53% and 39.27% were recorded in 2008/9 and 2009/10 in the plots treated with compound D + ammonium nitrate than in other plots.

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 (Figs. 5 A and B). At 6 weeks, the highest number of rotting tubers was recorded in tubers 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.

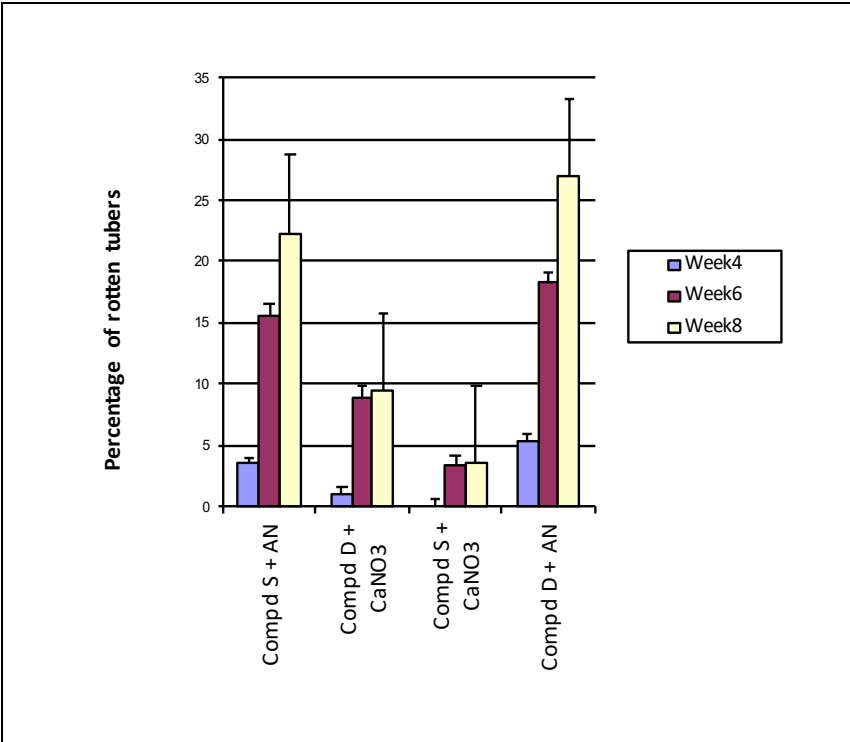
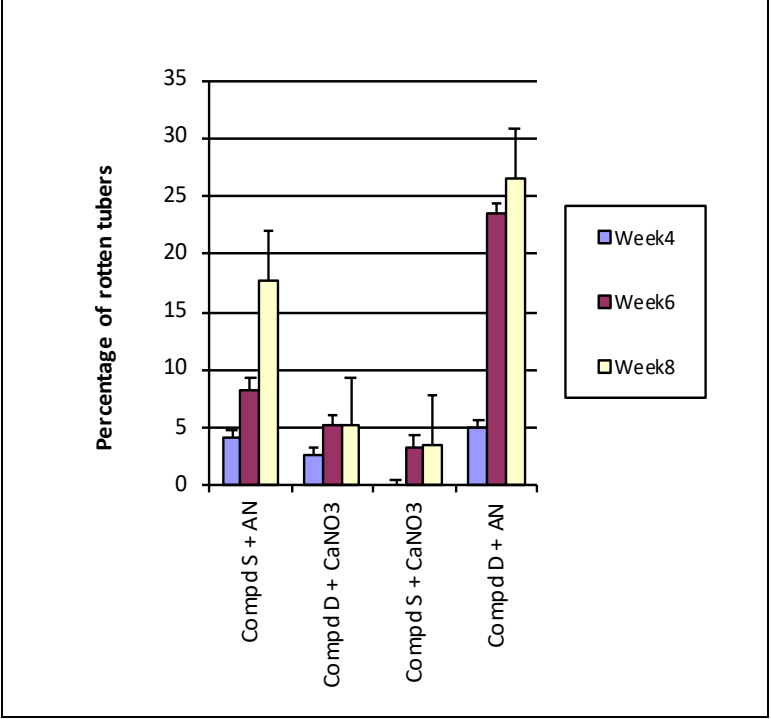


Figure 3: Number of tubers which rotted in storage at 4, 6 and 8 weeks after harvesting for experiment 1 (2008/9) (A) experiment 2 (2009/10) (B) ($P < 0.05$). The data represent the mean of three replicates and results were analyzed separately. Error bars on figures represent Standard Error of Difference (SED)



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 [19]. 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 [7, 8, 9].

Blackleg and soft rot incidence

Addition of calcium to the soil resulted in low blackleg and soft rot disease incidence and severity in the present study, showing that calcium is beneficial in increasing the resistance of potatoes to the two diseases. This supports the findings of McGuire and Kelman [7], who reported that potato varieties with low calcium levels in the tubers were more susceptible to the soft rot disease. A similar trend was also observed in this study although the concentration of calcium recorded for the plants in this study were higher than those recorded by Hélias *et al.* [16].

Soil nutrients

The differences in calcium concentrations might be due to agronomic practices at the experimental sites and the soil types used, in this study the researchers grew the potatoes in red fersiallitic soils with high CEC whereas other researchers [7, 20] used sandy soils with low CEC. Furthermore, different calcium formulations were also used in the two studies. McGuire & Kelman [7] used CaSO_4 as a source of calcium and in the present study the source of calcium was calcium nitrate. Environmental conditions in this study and those in plots used in other studies might have also contributed to the differences in calcium concentrations recorded in plant tissues of the two studies. Low blackleg and soft rot incidences recorded in this study 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 other researchers [10, 11] 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.

Reduced soft rot and blackleg incidences recorded in this study in the calcium treated plots clearly show the beneficial effects of calcium in managing the two diseases. 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 [19]. 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 defence mechanisms [20]. There was no correlation between soft rot incidence and blackleg incidence because the two disease symptoms were recorded on separate plants.

Blackleg and soft rot severity

High calcium content in host tissues has been correlated with increased resistance to several diseases [8, 21, 22]. This supports the results in this study which have reported that high calcium content in the potato tissues could have contributed to the reduced severity of both blackleg and soft rot diseases. Other studies have shown that plants grown in conditions of high Ca^{2+} content showed resistance to soft rot diseases [8, 20, 21]. The findings from these different studies support the results reported in this study. 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 [7, 8, 23]. Bateman & Lumsden [24] suggested that the excess calcium combines with pectin to form calcium pectate, which is resistant to the action of polygalacturonase (PG).

Soft rot losses in storage

The ability of *Pc.* subsp. *carotovorum* and other soft rot species to macerate plant tissue is dependent on their 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) [25]. These enzymes are crucial for the virulence of *Pectobacterium* subsp. and any mutations which affect production and secretion of these enzymes lead to reduced virulence [26, 27, 28]. These studies concur with the findings from the current research and the reduced post-harvest losses noted in this experiment could also be attributed to calcium interfering with the production of endopolygalacturonase. Increased calcium concentrations affect the gene which codes for the production of this enzyme. Endopolygalacturonase is the enzyme which is required in the early stages of infection. If calcium affects production of this enzyme, this could lead to reduced pathogen virulence [27, 29]. Increased extracellular calcium inactivates the gene which codes for endopolygalacturonase production but does not affect the production of other cell wall degrading enzymes [10]. Inactivation of a single gene encoding a particular pectic enzyme can drastically reduce virulence [27].

Yield assessment and yield losses

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 [3]. The average yield for BP1 is estimated to be at 30 t/ha [3]. 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 as compared to those grown in plots treated with compound S. These conclusions support the findings of Manzira [3], who recommended the use of compound S as a basal fertilizer for potato. Compound S also contains a higher content of potash, a nutrient which is required by potato during production especially at the tuber formation stage.



CONCLUSION

Several researchers have reported the beneficial effects of calcium in increasing potato resistance against soft rot pathogens [7, 8, 10, 11]. 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. Calcium also reduced post-harvest losses caused by the same pathogens. The results have also shown that compound S and Ammonium nitrate produce a higher yield and data from another experiment has shown that other forms of calcium can be used as supplement. This other experiment has shown higher yield and lower rot.

It will be beneficial to apply compound S fertilizer rather than compound D as the basal fertilizer when growing potatoes because application of compound S results in higher yields. The potato plants should be top dressed with calcium nitrate especially in areas where blackleg and soft rot diseases are prevalent as this fertilizer reduces incidence and severity of both diseases. Calcium nitrate also improves the shelf life of the potatoes and reduces post-harvest losses. Further research should be carried out to evaluate the effects of supplementing different soil types with calcium nitrate and different calcium sources should also be used in order to compare their effect on quality and yield of potato.

Table 1: Chemical composition of soils in the two experimental fields at the UZ campus taken in 2008/9 and 2009/10 prior to the experiment

Year	Field	pH	CEC Me/100g	CaO (ppm)	MgO (ppm)	K ₂ O (ppm)	Na (ppm)
2008	Block 1	6.0	5.6	402	105	24.3	33
2009	Block 2	6.7	4.9	126	35	29.4	27

CaO – calcium oxide, MgO – magnesium oxide, K₂O – potassium oxide, Na- sodium

CEC- Cation Exchange Capacity

Table 2: Soft rot incidence in mother and progeny tubers of BP1 at harvest for experiments 1 (2008/9) and 2 (2009/10)

Treatment	Experiment 1 (2008/9)			Experiment 2 (2009/10)		
	Rotten Mother Tubers (%)	Rotten Progeny Tubers (%)	Yield (tons ha ⁻¹)	Rotten Mother Tubers (%)	Rotten Progeny Tubers (%)	Yield (tons ha ⁻¹)
CompD+AN	41.1	16.53c	21.52b	39.7	30.13c	26.00b
CompS+CaNO ₃	36.1	6.53a	27.42c	36.1	3.50a	32.67c
CompD+CaNO ₃	37.2	5.21a	16.58a	35.0	6.44a	23.73a
CompS+AN	38.9	9.44b	33.87d	38.9	19.55b	39.27d
P-Value	0.370	<0.001	0.003	0.120	< 0.001	<0.001
SED	2.830	1.833	1.459	2.080	3.925	0.827
LSD (0.05)	NS	3.663	2.918	NS	7.850	1.654

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

AN – Ammonium nitrate, CaN – Calcium nitrate, CompD – compound D fertilizer, CompS – Compound S fertilizer

LSD- Least Significant Difference

SED- Standard error of difference

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