

**AN EVALUATION OF BLACK ROT (*Xanthomonas campestris* pv. *campestris*) DISEASE
TOLERANCE IN FOUR CABBAGE (*Brassica oleracea* var. *capitata*) VARIETIES.**

**A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE HONOURS IN
AGRICULTURE AND NATURAL RESOURCES**

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MAY 2014

DECLARATION

I.....do hereby declare that this is my original work done at Africa University, Mutare, Zimbabwe in partial fulfilment of the requirements for the Degree of Bachelor of Science (Honours) in Agriculture and Natural Resources and neither has it been submitted before nor is it being currently submitted to any university for the award of any other degree.

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ABSTRACT

Black rot (*Xanthomonas campestris* pv. *campestris*), is a major disease of the Brassica family throughout the world. In Zimbabwe the black rot problem is aggravated by cultivation of susceptible varieties by farmers and lack of technical expertise on disease management strategies. A field study was conducted at Africa University, Zimbabwe to evaluate tolerance of four cabbage (*Brassica oleracea* var. *capitata*) varieties that is Star 3301, Star 3311, Star 3316 and Star 3317, to black rot disease. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications and four varieties in each block. Each plot comprised 4 rows containing 8 plants in each row. Cabbage seedlings were transplanted on the 21st of January 2014. Compound D was applied as a basal dressing at the rate of 30g per plant station. At 21days after transplanting Ammonium Nitrate was applied at the rate of 2g per plant station. The seedlings were drenched using Carbaryl to control cutworms. Cultural practices such as sprinkler irrigation and weeding were carried out when necessary. Data on disease incidence and disease severity was collected from the 7th week after transplanting for four consecutive weeks. Bacterial Black Rot Disease Score Sheets on Brassicas were used for data recording as well as calculation of disease incidence and disease severity. Raw data on disease incidence was used to calculate Area Under Disease Progress Curve (AUDPC). Star 3311 had the least Area Under Disease Progress Curve (58cm²) while Star 3316 had the highest AUDPC (59.8cm²). Disease severity was low throughout the study period with a mean score of 3 for Star 3301, Star 3311, and Star 3316 while Star 3317 had a mean disease severity score of 4. Disease incidence was high throughout the study period reaching up to 100% in the 10th week after transplanting. Mean cabbage head weight was highest for Star 3311 and least for Star 3317. Area Under Disease Progress Curves and cabbage head weights were subjected to One-Way ANOVA Minitab Version. There was no significant difference among the four treatments at $p \leq 0.05$, meaning that they had the same black rot disease tolerance levels. Yield difference was found to be insignificant among the four varieties. Farmers can still achieve higher yields if Star 3311 is selected as it showed slightly higher resistance level when compared to the other varieties.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to Africa University for permitting me to do my research on the University farm. I am particularly indebted to the institution for providing me with library and internet facilities that helped to make my research a success. It is through the Jokomo Yamada Library that I was able to access resources such as journals and other useful textbooks for my research work. Through the institution's internet facilities, I was able to access online research material which was also very useful. Above all, I would like to extend my heartfelt gratitude to the Faculty of Agriculture and its staff compliment particularly Dr. Z. Chiteka the Faculty Dean, Messers C. Chikanda, and J. Tabarira for providing research materials and technical support respectively. A special word of thanks goes to my supervisor Dr. W. M. Manyangarirwa, who worked with me throughout the period of my research study. Last but not least my gratitude goes to the university farm labourers for helping as and when labour was required. The study was fully funded by Africa University through the Faculty of Agriculture and Natural Resources.

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DEDICATION

I dedicate this project to my beloved wife Agnes for her support and encouragement and my lovely daughters, Priscilla and Precious as well as my son Pride for their love and patience while I was studying at Africa University.

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CHAPTER ONE

1.0 INTRODUCTION

The bacterium *Xanthomonas campestris* pv. *campestris*, is the cause of black rot disease of crucifers. It is seed-borne and occurs worldwide (Williams, 1980; Alvarez, 2000). Black rot has become the major disease constraint to smallholder cabbage (*Brassica oleracea* var. *capitata*) growers in Africa where substantial crop losses are experienced especially during the warm and wet seasons (Day *et al.*, 1992; Onsando, 1992; Mguni, 1996; Massomo, 2002). The most economically important hosts of *Xanthomonas campestris* pv. *campestris* are the cole crops, forms of the polymorphic species, *Brassica oleracea* which include cabbage, cauliflower, broccoli, Brussels sprouts, and kale (Rubatzky and Yamaguchi, 1997). Bradbury, (1986), noted that *Xanthomonas campestris* pv. *campestris* is also reported on a number of other cruciferous crops, weeds, and ornamentals. Black rot has been reported as a major constraint in cabbage production in several countries including Nepal (Adhikari and Basnyat, 1999), Tanzania, (Black *et al.*, 2000), Zimbabwe (Mguni *et al.*, 1999) and in cauliflower in India (Sharma *et al.*, 1977; Sharma *et al.*, 1995). In Tanzania, black rot is wide spread in most of the cabbage growing areas, especially during the rainy seasons (Mgonja and Swai, 1998), where individual fields may succumb to heavy crop losses. The bacterium *Xanthomonas campestris* pv. *campestris* thrives especially well in warm and humid climates (Williams, 1980) and survives from season to season in infected seed (Walker and Tisdale, 1920) and in soil and even longer in plant debris in soil (Schaad and White, 1974) and it spreads readily to nearby plants by rain splash (Williams, 1980). Leaf invasions depend on water accumulating at natural openings, primarily hydathodes, where guttation droplets are formed during high humidity (Cook *et al.*, 1952; Robeson *et al.*, 1989). Typical disease symptoms are “V” shaped lesions starting from leaf margins with blackened veins and chlorosis of surrounding tissue (Sutton and Williams, 1970; Williams, 1980). Besides crop diversification and rotation, important ways to control black rot are the use of pathogen-free seed, seed treatment, sound sanitation practices including elimination of potential inoculum sources, such as infected crop debris (Williams, 1980; Kocks *et al.*, 1998), and weeds (Williams, 1980; Schaad and

Dianese, 1981), and use of resistant cultivars (Williams, 1980). Kocks and Ruissen (1996) showed that increased field resistance reduced the development of black rot in time and space in cabbage. Resistance to *Xanthomonas campestris* pv. *campestris* has been identified in different genotypes of *B. oleracea* (Bain, 1952; Williams *et al.*, 1972). Williams *et al.*, (1972) reported that most studies to identify useful resistance sources have been carried out at the seedling stage under greenhouse conditions, but a few have also included testing during the adult plant stage under field conditions. The study was conducted to evaluate the level of black rot (*X. campestris* pv. *campestris*) tolerance among the four cabbage varieties (Star 3301, 3311, 3316 and Star 3317) which were used in the study. The study was undertaken in the field conditions where natural bacterial infection occurred.

1.1 Statement of the problem

Bacterial black rot (*Xanthomonas campestris* pv. *campestris*) is among the major drawbacks of sound cabbage production. Bacterial black rot is one of the most destructive diseases in cabbage production globally. Huge losses are experienced by smallholder farmers in Africa where the disease occurs due to unreliable clean seed and high susceptibility of popular local varieties to black rot disease (Onsando, 1992). Williams, (1980) noted that black rot remains the greatest impediment to cabbage production and cultivation worldwide. In the humid tropics, the bacterium *Xanthomonas campestris* pv. *campestris* (Pam.) Dawson is devastating to cabbage and its relatives. This is due to cultivar susceptibility and environmental conditions that favour the pathogen (Staub and Williams, 1972; Ignatov, *et al.*, 1998 a, b). Varieties that are completely resistant have not been found but there are some varieties that are tolerant to black rot disease (Onsando, 1992).

1.2 Justification of the Study

Russell, (1898) suggested that black rot disease can be controlled using numerous measures some of which include use of pathogen-free planting material and the elimination of other potential inoculum sources that is infected crop debris and cruciferous weeds. Other measures cited include hot water seed treatment, fumigation of the seed beds as well as selecting sites that are well drained to reduce time that the seedlings are wet from dew or rain. Parsley, (1982) also suggested that black rot disease can be controlled using seed that has been treated in hot water to control seed –borne infection, preventing overcrowding of seedlings, choosing seedling production areas free from susceptible weeds, ploughing in diseased crops after harvesting, crop rotation, weeds and insects control as well as applying recommended chemicals to limit spread of black rot especially in seedlings.

Despite these procedures, black rot remains the most important disease of crucifers worldwide (Williams, 1980; Mguni, 1996). Massomo, (2002) stated that the use of bactericides is expensive to smallholder farmers and they are not environmental friendly hence pose a threat to human health after consumption. Host plant resistance remains the cheapest strategy in the control of the disease. Evaluating levels of tolerance among four cabbage cultivars used in the study will provide a more natural means of curbing black rot disease problems in cabbage production. The study will therefore help to identify which of the four cultivars used in the study is most tolerant and most susceptible so that recommendations can be drawn for farmers on cultivar selection.

1.3 Main Objective

The overarching objective of the study was to have a better understanding of the seasonal occurrence of diseases of cabbage (*Brassica oleracea* var. *capitata*) in the small and large scale farms of Zimbabwe with emphasis on black rot (*Xanthomonas campestris* pv. *campestris*). A secondary objective was to understand the differences in tolerance of four cabbage (*B.oleracea* var. *capitata*) varieties to black rot disease and to generate information that can be used by farmers and crop protection practitioners in selecting tolerant varieties of cabbage.

Specific Objectives

Objective 1

To determine the seasonal occurrence of black rot (*Xanthomonas campestris* pv.*campestris*) on cabbages (*Brassica oleracea* var. *capitata*) in Zimbabwe.

Rationale

An understanding of the seasonal occurrence of black rot (*Xanthomonas campestris* pv.*campestris*) on cabbage (*B.oleracea* var.*capitata*) provides farmers and crop protection practitioners with information relevant to the control of the disease as well as proper timing of cabbage production.

Objective 2

To determine the relative tolerance of four cabbage varieties that is Star 3301, Star 3311, Star 3316 and Star 3317 to black rot (*Xanthomonas campestris* pv. *campestris*) disease.

Rationale

An understanding of the tolerance levels among the four different cabbage varieties to black rot disease attack provides growers and crop protection practitioners with knowledge on variety selection to minimize losses incurred due to black rot disease.

1.4 Hypothesis

The four cabbage Starke Ayres Star varieties show significant differences in Bacterial black rot disease tolerance

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of cabbage production

According to Swiader and Ware, (1994) cabbage is the most important member of the cole crops. The plant is grown for its fleshy leaves, which may be served boiled, raw (cole slaw), or fermented (sauerkraut). It is more tolerant of warm temperatures than other cool season cole crops and can be grown in summer in many regions. Cabbage is fairly nutritious and ranks higher than tomatoes but lower than spinach in mineral content. Swiader and Ware, (1994) reported that cabbage is classified as *Brassica oleracea* (capitata group). For more than 3000 years, cabbage has been used as a food crop although initially it was probably used for medicinal purposes. The ancient Greeks held it in high esteem and their fables claim its origin from the father of their gods. Present day cultivars most likely originated from wild-non-heading types found along the chalky coasts England and North-western France, where the Romans or Celts may have introduced them from the coastal regions of Mediterranean Sea. Cabbage was common throughout Europe by 900 A.D and was introduced into the United States around the 16th century by the early colonialists (Swiader and Ware, 1994).

2.2 Botany of Cabbage

Nonnecke (1922) noted that cabbage is a biennial plant. The first year of growth produces the head. Where winter killing is a problem, the heads with the roots attached are stored over winter and replanted for the final phase of flowering and seed production. If not damaged in the transplanting process, the cabbage plant will develop a strong tap root system supported by a wide net work of fibrous feeder roots located in the top 22cm of the soil with greatest concentration just below the surface. As the cabbage plant develops, it initially rosettes until a basic number of leaves are formed. The terminal bud at this point then begins to grow, forming an ever-increasing overlap of leaves that eventually become the head (Nonnecke, 1922).

2.3 Climatic requirements

Cabbage is grown throughout the year but for best results, cool moist seasons are preferred. Mature plants can tolerate temperatures of -3°C for a very short time but several days of cold temperatures in the region of -1°C to 4°C will induce bolting and production of seed (Gilmour, 1983). The average optimum temperature is approximately 18°C with a maximum of 24°C and an average minimum of 4.5°C (Thompson, 1939). Japanese investigations have shown that the optimum temperature for growth and heading is between 15°C and 20°C.

Above 25°C, growth is arrested while the minimum temperature for growth is just above 0°C (Bose and Som, 1993). Bursting of the head is encouraged when very wet conditions follow very dry periods. Consequently irrigation is essential to avoid this condition and ensure more even and uninterrupted growth. After a dry period during a hot period, a distastefully strong cabbage flavour may develop (Roberts *et al.*, 1972). Varieties tolerant to hot conditions are therefore useful and examples are cape spitz and Co cross. Under warm summer conditions, the plants are more susceptible to aphid infestation and attack by the cabbage moth caterpillar, both of which require regular and thorough control. Bacterial black rot is also prevalent during summer (Thompson, 1939).

2.4 Soil requirements

According to Gilmour (1983), cabbages will grow in most well-drained soils provided they are adequately fertilized and well supplied with organic manure. Thompson and Kelly (1975) suggested that slightly acidic soils are preferred with a pH (Ca Cl₂) reaction of 5.5 to 6.1. Lime should not be applied unless the pH drops to below the lower value. If lime is necessary it should be applied at least one month before planting. Lands should be well ploughed and thoroughly prepared and cultivated to obtain a good tilth for planting (Ware and McCollum, 1975).

2.5.0 Management of cabbage crop

Rapid and continuous growth should be encouraged (Roberts, *et al.*, 1972). Cultivation should be shallow since most roots are in the top 300mm but not more often than is necessary to keep weeds under control, especially during the early stages. Herbicides may be used to control weeds under summer rainfall conditions. Trifluralin is used at the rate of 2l/ha, cross disced into the soil before transplanting. This chemical controls annual and certain broad-leaved weeds and is effective for approximately four months. Lasso, applied at the rate of 4l/ha may also be used. It is applied after the first transplanting irrigation but before weed emergence. The chemical controls broad-leaved weeds for six to eight weeks (Gilmour, 1983).

2.5.1 Fertilizer management

Cabbages are relatively gross feeders and so require adequate levels of fertilizer and manure (Gilmour, 1983). A fertilization programme should be based on the results of a soil analysis. As a general guide 25 to 50t of organic manure should be applied and incorporated before planting. According to Gilmour (1983), the fertilizer requirements are 55-90kg/ha Nitrogen, 110-180kg/ha P_2O_5 and 55-90kg/ha K_2O . A top dressing of 100kg/ha should be applied at 3 weekly intervals after transplanting. Alternatively, compound S (6:17:6 +0.4B +9% S) can be used as a base dressing at a rate of 1000kg/ha in addition to the Nitrogen top dressing. If fertilizer is to be banded, one half of the required fertilizer should be broadcast and disced into the top 180-250mm of soil, the remainder should be placed in bands at transplanting time, 75-100mm to each side of the row and 100mm deep. Boron deficiency is unlikely if compound S is used but if straights are used and should boron be deficient hollow stems and brown necrotic areas result. Fertilizer borate can be applied at the rate of 30-35kg/ha. Magnesium deficiency should not be a problem if dolomitic limestone is used (Anonymous, 1955).

2.5.2 Production of Brassicas in Africa

A wide range of Brassica crops are grown in Africa, though kales and cabbages are generally the most important crops in terms of quantity of production. Precise data is not easy to come by except where focused studies have been carried out for example in Kenya and Ghana. In Kenya the estimated annual production of Brassicas is 550 000 t, with 95% of the production in the highlands on 35 000 ha (FAOSTAT, 2007). In East Africa, about 90 % of the Brassica production is by smallholder on plots of 0.1-0.5ha and this probably applies to other regions of Sub Saharan Africa (SSA), although in South Africa larger scale commercial production is also important (Kfir, 2004). Brassicas are particularly important in the peri-urban farming sector in East Africa (Oruku and Ndun'gu, 2001) as they are key components of the local diet and nutritionally very important for poorer people who cannot afford alternative vegetables. Kales in particular are a major item in the local diet and are an important smallholder subsistence crop in Kenya, Ethiopia, Zimbabwe and Mozambique (Lo'hr and Kfir, 2004). The production of cabbage and open leaf kales for local markets and regional trade is predominant, although commercial production of spinach, broccoli and pak choi for export is increasing, especially in Kenya and Tanzania (Nyambo and Lo'hr, 2005). Production is mainly confined to the cooler wet seasons but there are areas where dry season production is practiced, for example, in export operations that may continue year round. While the highlands remain the focus of production, some production has started in semi-arid lowlands (Nyambo, 1995). In Ghana and Benin, production of cabbages for the peri-urban market is very important, with production concentrated in the lowland zones during the wet season when pest pressure is low (Cherry, 2004) In West Africa as a whole, Brassicas are important vegetables grown in an area of 13 900 ha with annual production of 140 500t (FAOSTAT, 2003).

2.6 Constraints to cabbage production

There are a number of constraints and among them insect pests and diseases are the most problematic in cabbage production.

The diamond back moth (DBM), *Plutella xylostella* (L), remains a major pest of Brassica crops worldwide and has been estimated globally to cost US\$1 billion in direct losses and costs (Shelton, 2007). Diamond back moth is cosmopolitan in its distribution, occurring in all major zoogeographical regions of the world wherever crucifer crops are cultivated (Talekar and Shelton, 1993). Control of Diamond back moth is mainly through use of insecticides (Diaz-Gomez *et al.*, 2000). Due to development of resistance to the insecticides by DBM, other methods used to control it include cultural practices through diversification of vegetation (Hooks and Johnson, 2003). Where the pest has developed resistance Integrated Pest Management (IPM) can be adopted (Manyangarirwa, 2009).

Cabbage is also affected by diseases such as black leg, caused by a fungus (*Phoma lingam*) in which symptoms are in form of lesions on the stem near the soil surface which become sunken and dark, and may girdle the stem. Black ring spot which is caused by a virus results in necrotic, dark and often sunken rings on the leaf surface. *Fusarium oxysporum* which is a fungus is responsible for disease called cabbage yellows or wilt and yellowing and dwarfing of cabbage are some of the symptoms (Gilmour, 1983). Black rot disease which is caused by the bacterium (*Xanthomonas campestris* pv. *campestris*) is a disease of major economic importance to both commercial and smallholder farmers (Gilmour, 1983).

2.7.0 Bacterial black rot (*Xanthomonas campestris* pv. *campestris*) (Pammel) Dowson disease of brassicas

Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson which is a rod shaped gram negative bacterium is the most serious disease of crucifers worldwide; it attacks all cultivated varieties that grow in temperate and subtropical zones where rainfall or heavy dews are plentiful and where average temperatures are between 15°C and 21°C. Black rot was recognized first in Kentucky in 1889 and soon after was reported worldwide (Sherf and Macnab, 1986). Cauliflower and cabbage are the most readily infected of the crucifers, although kale is almost equally susceptible. Broccoli and Brussels sprouts are intermediate, and radish is quite resistant to most but not all strains (Cook *et al.*, 1952).

Other susceptible crucifers are kohlrabi, Chinese cabbage, rutabaga, turnip, collards, rape, charlock, field mustard, black mustard, and shepherd's purse. A closely related bacterial species can affect horseradish, radish, stock, and winter cress. Losses from black rot generally are high because of the rapidity of spread when weather conditions favour pathogen growth and dissemination. Soft rot bacteria enter black rot lesions, move into the heads, and render the heads worthless. Late infections may merely spot the leaves or may result in smaller heads (Sherf and Macnab, 1986).

2.7.1 Symptoms of Bacterial black rot

Sherf and Macnab, (1986) observed that the plants can be affected at any growth stage. On young plants cotyledon margins turn black, and cotyledons later shrivel and drop off. True leaves also can be infected at the margins; the first symptom being a small wilted or yellow V-shapes area at the leaf margin. As the diseased area enlarges, internal tissue nearby turns black or brown. Large yellow splotches or sectors at leaf margins are noticeable in the field, and these usually are most prevalent in low areas and along windbreaks where plants remain wet for long periods (Williams, 1980). On cauliflower, numerous minute brown specks resembling peppery leaf spot may appear. Entire leaf turns yellow or wilts and finally drops to the ground. Occasionally, affected plants have a long bare stalk with only a small tuft of leaves at the top. When affected stems are cut crosswise, a black vascular ring may be evident where bacteria have moved into water ducts. In a similar manner, the leaf petiole and the veins show blackened dots. The black vascular ring and darkened petioles closely resemble symptoms of *Fusarium yellows*, except that the black rot bacteria occasionally cause slight pockets of infection outside the vascular ring. In kohlrabi and radish, vascular discoloration sometimes cannot be detected until the edible parts are cut open. Occasionally, there are years when the disease organism seems to remain dormant in the host until favourable weather occurs in the autumn; then symptoms appear. The heads from late infected plants can develop black vascular symptoms during winter storage. A semi-selective medium has been developed that may help in diagnosis where isolations can be made (Cook *et al*, 1952).

2.7.2 Black rot disease cycle

The pathogen lives over winter on and in seed and in debris from infected plants left in the field, particularly in surviving cabbage or Brussels sprouts plants, as well as in plants stored for seed production (Sherf and Macnab, 1986). Recent work has demonstrated that weeds can harbour the black rot bacteria including the *Brassica campestris*, *B. nigra*, *B. geniculata*, *Lepidium virginicum* and *Cardaria pubescens*. The bacteria have been shown to be spread as far as 11.9 m into cabbage fields (Schaad and Kendrick, 1975). In the spring, when seedlings emerge, bacteria pass from cotyledons into young leaves, either directly or through stomata. Bacteria move downwards through the water ducts until they reach the stem, from which they migrate to roots and leaves. Later inoculations take place through hydathodes along leaf margins, through insect feeding injuries, and in very susceptible crops such as cauliflower, directly through stomata. The bacteria are spread by splashing or running water, by blowing of detached leaves, or by handling infected plants (McGrath, 1994). Insects can carry the bacteria between plants. Seed borne bacteria can be disseminated worldwide. The optimum temperature for pathogen growth is from 26°C to 30°C, the minimum being 5°C and the maximum being 36°C (Schaad and White, 1974).

2.7.3 Control of black rot disease

Black rot usually is introduced to a farm via infected seed or transplants. Once a trace of disease is present in a seedbed, crowded plants provide conditions that are ideal for bacterial spread to thousands of seedlings nearby (Sherf and Macnab, 1986). Seed lots must be entirely free of bacteria before planting. Hot water soak-treatment for cabbage and Brussels sprouts at 50°C for 25 minutes, cauliflower and broccoli 50°C for 20 minutes, effectively remove the bacteria from minimally infected seed lots. Schaad has developed a laboratory assay for detecting small amounts of seed infection in a seed lot (Schaad and Kendrick, 1975). Humaydan *et al*, (1980) reported that a new combination treatment using antibiotics,

sodium hypochlorite, and Thiram is promising for control of both Black rot and Phoma black leg. When one of these treatments is used on fresh seed, germination reduction is minimal.

Plant bed and field soils must have been free of crucifer crops for at least 2 years, because the bacteria can persist at least one winter in debris from diseased plants. Direct seeding should be used where feasible. Schaad and White, (1974) reported that crucifer plantings should be located where air and soil drainage is good. Transplants should not be dipped into water before transplanting. Good sanitation practices involving quick plough down of crucifer crop debris and the use of clean containers and equipment are important. If traces of black rot are detected early in production fields, several applications of fixed copper may reduce the disease spread (Walker, 1923).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Trial site

The study was conducted at Africa University specifically in the farm research block (18°53'70.3"S and 32°36'27.9"E). Africa University is located about 17km from Mutare along Nyanga Road, in Manicaland Province of Zimbabwe, in Agro-ecological Region II. Africa University farm is located at altitude 1,131m Above Sea Level. The average annual temperature is 19°C and the coldest month is July where minimum temperatures drop to 6°C with maximum temperatures reaching 20°C. The hottest month is October in which the minimum temperature is 16°C with maximum temperature of 32°C. The annual rainfall for Mutare averages 818mm and most of the rains fall between December and February with the rain season lasting in April (Retrieved from :<http://en.wikipedia.org/wiki/Mutare>). The soils at the University farm research block are mainly red loams.

3.2 Field experimental set up

Cabbage seedlings of the cultivars; Star 3301, Star 3311, Star 3316 and Star 3317 were sown in the green house at Africa University on 29th of December 2013 and transplanted into the field plots on January 21st, 2014. A basal dressing of Compound D was applied at the rate of 30g per plant station. The experimental design that was used for the study was the Randomised Complete Block Design (RCBD) comprising three replications. Each block or replication comprised 4 plots of the four different varieties with each plot comprising 4 rows. Each row was made up of 8 plants to give a total of 32 plants per plot. The spacing between the blocks and the plots was 1m and the seedlings were transplanted at a spacing of 50cm by 50cm. Overhead irrigation was applied immediately after transplanting and the seedlings were drenched using Carbaryl a week after transplanting. The initial top dressing of Ammonium Nitrate was applied at 21 days at the rate of 5g per plant.

The subsequent applications of top dressing were done at two- week intervals. Carbaryl was applied to control diamond back moth after noticing windowed leaves. Weeding was done when it was necessary.

3.3 Trial layout

The trial was laid down in the physical layout shown in table 3.1 and plate 3.1

Table 3.1: Trial layout

BLOCK 1	BLOCK 2	BLOCK 3
Star 3301	Star 3317	Star 3311
Star 3311	Star 3316	Star 3317
Star 3316	Star 3311	Star 3301
Star 3317	Star 3301	Star 3316



Plate: 3.1 Field layout of the trial showing three blocks, with block 2 in the centre.

3.4. Data Collection

Data on disease incidence and disease severity was collected from the 7th week after transplanting for four consecutive weeks up to the 10th week after a bacterial ooze test was conducted in the laboratory to confirm the disease on the cabbage leaves. Data was only collected from the net plot and outside lines were discarded (Plate 3.4). Disease severity was determined using the severity scale of 0 to 9, and 0 denoted no disease on an increasing scale with 9 denoting whole plant dying. Some disease scoring sheets were used to record bacterial black rot disease incidence; disease severity and disease index (see Appendix 1 and 2). Raw data on disease incidence was presented on a table (see appendix 2). Data on disease incidence was used to calculate Area Under Disease Progress Curves (AUDPC). The Area under Disease Progress Curve for each treatment was then subjected to ANOVA on Minitab version.

3.5 Data analysis using MINITAB

Using MINITAB version to analyse the data, each replication was entered as the factor in column C1 and output of the disease incidences (Calculated using the Area Under Disease Progress Curves) was assigned column C2. Analysis was done using One-Way ANOVA at $p < 0.05$. Data for cabbage head weights was entered into MINITAB version after which analysis was done using One-Way ANOVA.



Plate 3.4. Data collection in the 10th week after transplanting

CHAPTER FOUR

4.0 RESULTS

4.1 Disease confirmation

In the 7th week after transplanting, some water soaked lesions (Plate 4.1) were noticed on one of the leaf of the variety Star 3316 in the field and a Bacterial Ooze test was conducted in the Laboratory. A 2cm by 2cm specimen of the affected area was made and observed under the stereo microscope for fruiting bodies. After no fruiting bodies were observed, a 3mm by 3mm specimen was cut and a slide was prepared and a bacterial ooze test was conducted on the compound microscope and bacterial ooze was observed. Based on the confirmation of the disease, data was then collected up to the 10th week after transplanting.



Plate 4.1. A “V” shaped lesion showing Bacterial Black Rot Disease symptoms on Star 3316

4.2 Disease Incidence for the four treatments

In the 7th week when data collection started, all plots showed symptoms of the black rot disease especially on the cotyledons. At least 96% of the plants in the net plot had symptoms of the disease on the cotyledons on the variety Star 3301. Star 3311 had 94% of the plants in net plot affected by the disease while variety Star 3316 had 97% incidence. Variety Star 3317 had 97.3 % incidence in the 7th week from transplanting. In 8th week, when second readings were taken, variety Star 3301 and Star 3316 had all plants in the net plot affected by the disease and incidence rose to 100% while Star 3311 and Star 3317 had 96% and 97.3% disease incidence respectively. Readings from the 9th and 10th week showed disease incidence of 100% across all the four treatments. Results showed that Star 3301 and Star 3316 had higher susceptibility levels (99% and 99.4% respectively) among the four treatment while Star 3311 showed that it was the most tolerant (97.5%) to black rot disease of the four varieties though there was no significant difference among the four varieties. Star 3317 had intermediate susceptibility level of 98.7% disease incidence. There was no significant difference among the four varieties (see Appendix 5) in terms of black rot disease tolerance ($p > 0.05$).

Table.4.1: Bacterial Black rot Disease Incidence table for the four treatments

Week	Star 3301	Star 3311	Star 3316	Star 3317
1	0%	0%	0%	0%
2	0%	0%	0%	0%
3	0%	0%	0%	0%
4	0%	0%	0%	0%
5	0%	0%	0%	0%
6	0%	0%	0%	0%
7	96%	94%	97.6%	97.3%
8	100%	96%	100%	97.3%
9	100%	100%	100%	100%
10	100%	100%	100%	100%

Table 4.2: List of cabbage cultivars used in the study indicating predicted and actual observed levels of resistance.

Variety	<u>Level of Resistance</u>		Source (Country/ Seed Company)
	Predicted ^a	Observed ^b	
Star 3301	Tolerant	Tolerant	South Africa/ Starke Ayres
Star 3311	Highly Tolerant	Highly Tolerant	South Africa/ Starke Ayres
Star 3316	Tolerant	Susceptible	South Africa/ Starke Ayres
Star 3317	Highly Tolerant	Tolerant	South Africa/ Starke Ayres

^a Variety Resistance described as tolerant/ highly tolerant/ susceptible by parent Seed Company (Retrieved from: <http://www.starkeyayres.co.za/factsheet>).

^b Studies conducted at Africa University based on observations of the Area Under Disease Progress Curves (AUDPC). Highly tolerant variety is based on least Area Under Disease Progress Curve.

4.3 Rainfall pattern during the study period

The rainfall regime is shown in figure 4.1. Bacterial black rot disease is favoured by warm moist conditions. The disease thrives well when relative humidity is around 80-100%. The month of February received a total rainfall amount of 156mm, while March and April received 53mm and 20mm respectively. Much of the rainfall for the 2013/2014 season was received during the month of December (264mm) when the seedlings were still in the green house. High rainfall during the study period was recorded during the month of January (181mm) before the seedlings were transplanted. The rains that fell in February led to bacterial black rot disease symptoms spreading quickly during the month of March when the symptoms were first observed

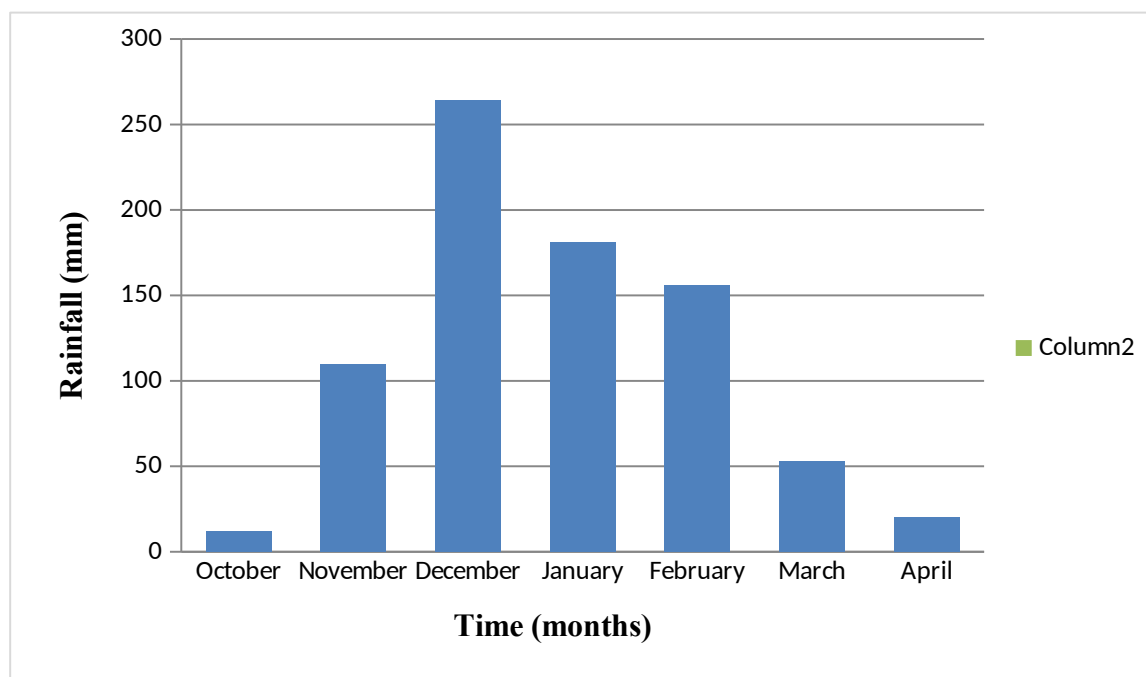


Figure 4.1: The pattern of rainfall during the period of evaluation

4.4 Disease severity among the four varieties

Disease severity was calculated for all the varieties and a table of results were presented. The disease was not severe as all the four treatments had disease severity less than 50%. The mean disease severity score was 3 for the varieties; Star 3301, Star 3311, Star 3316 while the mean score for Star 3317 was found to be 3.3 (see Appendix 3). The results showed that only 25% of the plant tissue was affected by the disease even though disease incidence was too high. The results also showed that there are no significant differences among the four varieties as regards disease severity. The treatments showed the same level of tolerance to bacterial black rot disease of Brassicas.

4.5 An assessment of head weights of the four varieties

At harvest the cabbage heads were collected and weighed individually using the sensitive balance scale to get the yield per variety that was later converted to t/ha. Star 3311 gave the highest mean head weight of 3.9kg with frame leaves, while Star 3301 gave mean head weight of 3.7kg with frame leaves.

The least mean head weight from Star 3317 at 3.23kg. Star 3316 had a mean head weight of 3.58kg with frame leaves.

Table 4.3: Mean cabbage head weights and estimated yields (t/ha) for the four varieties.

Variety	Mean Head Weights (kgs)	Plant Population Per hectare	Estimated yields (t/ha)
Star 3301	3.7	40 000	148
Star 3311	3.9	40 000	156
Star 3316	3.58	40 000	143.2
Star 3317	3.23	40 000	129.2

CHAPTER FIVE

5.0 Discussion

The varieties of cabbage studied showed tolerance to bacterial black rot disease as they had a low disease severity score (Appendix 3). Star 3301, Star 3311, and Star 3316 had an average score of 3 (25 % of plant tissue affected by disease) whereas Star 3317 had a mean score of 4 (40% of the plant tissue affected by the disease). Variety Star 3311 showed a slightly higher level of tolerance compared to other varieties studied. The variety had the least Area Under Disease Progress Curve (58 cm²) compared to 59.2 cm², 59.8 cm², and 59.2 cm² for Star 3301, Star 3316 and Star 3317 respectively as stated by the literature from the Seed Company (Table 1). In terms of disease incidence there was no significant difference among the four treatments as percent disease incidence was well in the same range from the first week (7th week) of data collection. The least disease incidence was recorded for Star 3311(94%) while the highest was recorded for Star 3316 (97.6%). In the second week of data collection that is 8th week after transplanting the percent disease incidence rose to 96 % for Star 3311, while for Star 3301 and Star 3316, it rose to 100% with Star 3317 recording 97.3%. In the 9th and the 10th week disease incidence rose to 100% for all the treatments to mean that all the plants in the net plot showed symptoms of bacterial black rot disease.

In Tanzania, black rot is wide spread in most of the cabbage growing areas, especially during the rainy seasons (Mgonja and Swai, 1998), where individual fields may succumb to heavy crop losses. The bacterium *Xanthomonas campestris* pv. *campestris* thrives especially well in warm and humid climates (Williams, 1980) and survives from season to season in infected seed (Walker and Tisdale, 1920) and in soil and even longer in plant debris in soil (Schaad and White, 1974) and it spreads readily to nearby plants by rain splash (Williams, 1980). The bacteria are spread by splashing or running water, by blowing of detached leaves, or by handling infected plants (McGrath, 1994). Insects can carry the bacteria between plants. Seed borne bacteria can be disseminated worldwide. (Williams, 1980). Leaf invasions depend on water accumulating at natural openings, primarily hydathodes, where guttation droplets are formed during high humidity (Cook *et al.*, 1952; Robeson *et al.*, 1989).

According to Miller *et al* (1996) bacterial black rot disease is most severe when heads are forming and may lead to prevention of head formation which results in the yield losses. This explains why the symptoms developed in the 7th week after transplanting when cumulative rainfall figures were increasing.

The disease causing agent (*Xanthomonas campestris* pv *campestris* (*Xcc*) survives very well especially in cabbage and Brussels sprout refuse, in numerous weeds including black mustard, field mustard, shepherd's purse, Virginia pepper weed and cress (McGrath, 1994).

None of these weeds were found in the trial site. The dominating weeds in the trial plot was found to be *Galinsoga parviflora*, *Commelina benghalensis*, and *Bidens pilosa* and do not harbour the pathogen.

Pathogen thrives well in free moisture in the form of dew, fog or rain which is required for infection and disease development. The pathogen is spread from plant to plant by splashing rain and does not usually spread in dry weather (Schaad and Dianese, 1981). Rainfall soon after transplanting was not evenly distributed and at one point the pump at the farm was down such that irrigation was not possible. This resulted in periodical moisture stress to the newly transplanted seedlings. This in a way explains why the symptoms were first observed in the 7th week after transplanting as conditions for the pathogen were sometimes dry and unfavourable. According to Day *et al* (1992), the disease is usually prevalent in low lying areas where plants remain wet for long periods. The site that was chosen is not low lying and had North facing slope. The disease also favours poorly drained soils for it to thrive. The soils at the trial site are mainly loams which are well drained and disease inoculum was very low.

The rainfall pattern was such that most of the rains fell in the months of November, December and January (110mm, 264mm, 181mm) respectively while the seedlings were still to be transplanted. In February total rainfall received was 156mm and the rains started to dwindle and in March 53mm of rainfall was received. In April the rains were only received after the last data was collected. Dry conditions prevailed for much of the study period and symptoms were developing on the cotyledonous leaves which were only visible in week 7th after transplanting which might have an influence on the results of the evaluation. The disease symptoms then spread onto the outer leaves.

Disease severity remained very low throughout the evaluation period as shown by the disease severity mean scores of 3 or 25% plant tissue affected by the disease for Star 3301, Star 3311, and Star 3316 while the mean disease severity score was a bit higher for Star 3317 which had 4 or 40% plant tissue affected. However, disease incidence proved to be very high across all the net plots with all of the plots showing at least 25% plant tissue affected by the disease throughout the study period (Appendix 3). Figures obtained from the Area Under Disease Progress Curve showed that Star 3316 had the highest (59.8 cm²) while Star 3311 had the least Area Under Disease Progress Curve (58cm²). The results showed that Star 3311 was highly resistant while Star 3316 was the most susceptible of all the treatments.

Results of cabbage head weights analysis showed a higher mean head weight for Star 3311 (3.9kgs) while the least mean head weight was recorded for Star 3317 (3.23kgs) while the other two varieties (Star 3301 and Star 3316) recorded mean head weights of 3.58kgs and 3.7kgs respectively. The mean head weights obtained seem to tally with predicted mean head weights from the seed company. Star 3301 should yield mean head weight of 4.0-5.0kgs, Star 3311 should have 2.5-3.0kgs while Star 3316 and Star 3317 should have mean head weights of 3.0-5.0kgs and 2.0-3.5kgs respectively (Retrieved from <http://www.starkeyayres.co.za/factsheet>). When converted to t/ha, Star 3311 had the highest yield of 156t/ha while Star 3317 had the least yield of 129.2t/ha at an estimated plant population of 40 000 heads per hectare per variety. Star 3301 and Star 3316 had yield of 148t/ha and 143.2t/ha respectively. The results show that the Starke Ayres Star varieties are tolerant to black rot disease as there was no significant difference between the mean head weights predicted by the seed company and those observed in the study at Africa University farm.

The ANOVA analysis that was done on Minitab version (Appendix 5) showed that in terms of disease incidence there was no significant difference at $p > 0.05$. The lines of the One-Way ANOVA analysis showed an overlapping pattern denoting that there was no significant difference in tolerance levels to bacterial black rot disease ($p > 0.05$) even though the variety Star 3311 had the least Area Under Disease Progress Curve (AUDPC). One-Way ANOVA analysis of the head weights showed no significant

difference ($p > 0.05$) among the varieties confirming that all the four varieties are tolerant to black rot disease. The hypotheses that there was significant difference among the four Star varieties evaluated was not true. In general all the varieties used in the study showed tolerance to the disease as predicted by the seed company. This explains the reason why the varieties were not significantly different at $p \leq 0.05$. The disease severity was not high because only certified seed was used and yields were not much affected by the high disease incidence.

CHAPTER SIX

6.0 CONCLUSION

Conclusions can be drawn that the varieties used in the study are all tolerant to bacterial black rot disease as indicated by the results of the One-Way ANOVA analysis. The One-Way ANOVA analysis showed some overlapping pattern in the lines indicating that there was no significant difference in tolerance levels of the four treatments. Star 3311 had disease severity mean score of 3 and the least Area Under Disease Progress Curve (AUDPC of 58cm²) and was the most tolerant of all the four varieties used. The variety also gave the highest mean head weight despite having a higher level of bacterial black rot disease incidence. The varieties showed high level of tolerance at the seedling stages from 1st week to the 7th week after transplanting. The disease is more severe at head formation and this is why symptoms showed up in the 7th week from transplanting. The pattern of rainfall and periodic dry spells during the evaluation period also had an effect on the spread of the disease as the pathogen favours wet and rainy conditions. Host plant resistance remains the cheapest strategy in the control of the disease as it gives yield benefits by reducing crop losses in the rainy seasons. Where testing is continuously being carried out on bacterial black rot resistant varieties and the dissemination of information carried out, farmers can benefit from the developed varieties.

6.1 Recommendations

1. The study demonstrated that farmers can grow bacterial black rot tolerant varieties such as Star 3311 which is highly tolerant to the disease.
2. Farmers can adopt cultural control method for the disease for instance crop rotations and maintaining the field weed free.
3. Trials for evaluating cabbage varieties for black rot tolerance should be done such that it coincides with peak of the rainy season.

4. The study should include other varieties from different seed companies in the study as the Starke Ayres varieties used for study purpose demonstrated the same tolerance levels.
5. The pathogen causing bacterial black rot overwinters in seed, on seed or in plant debris; hence there is need for the farmers to destroy plant debris after harvesting cabbage by ploughing.
6. Study should be carried out to evaluate if there is correlation between black rot disease and yield stability.

CHAPTER SEVEN

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APPENDICES

Appendix 1: Bacterial Black Rot Disease Score on Brassicas

Date.....

Trial.....

Plot Number.....

Treatment.....

Plant Number	Disease Incidence	Disease Severity	Disease Index (Incidence x Severity)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
Mean			

Scoring Scale:

0 = No Disease

3 = 25 % of cover leaves infected

5 = 50 % of cover leaves infected

7 = 75 % of cover leaves infected

9 = Whole plant dying

Appendix 2: Bacterial Black Rot Disease Incidence on Brassicas

Date.....

Trial.....

Plot Number	Treatment	Plant Count in Net Plot	Number of Infected Plants	Percent Incidence
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
Mean				

Comment.....

.....

Appendix 3: Disease Severity Table in Scores

	Star 3301			Star 3311			Star 3316			Star 3317		
Weeks	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
7	2	2	2	2	2	2	2	2	3	2	2	2
8	3	2	3	3	3	3	3	3	3	3	2	4
9	3	3	3	3	3	3	3	3	3	3	3	4
10	3	3	3	3	4	4	4	3	4	4	3	4
Mean Score	3	3	3	3	3	3	3	3	3	3	3	4

Appendix 4: Area Under Disease Progress Curves Table (cm²)

Treatment	Star 3301	Star 3311	Star 3316	Star 3317
Rep 1	60 cm ²	59.4 cm ²	60 cm ²	60 cm ²
Rep 2	60 cm ²	54.8 cm ²	59.3 cm ²	57.7 cm ²
Rep 3	58.8cm ²	60 cm ²	60 cm ²	60 cm ²
Mean	59.6 cm ²	58 cm ²	59.8 cm ²	59.2 cm ²

Appendix 5: ANOVA ANALYSIS (One-Way ANOVA: C2 versus C1) for disease incidence

Analysis of Variance for C2

Source	DF	SS	MS	F	P
C1	3	5.29	1.76	0.67	0.593
Error	8	21.00	2.63		
Total	11	26.29			

Individual 95 % CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-+-----+-----+-----+-----
1	3	59.600	0.693	(-----*-----)
2	3	58.067	2.845	(-----*-----)
3	3	59.767	0.404	(-----*-----)
4	3	59.233	1.328	(-----*-----)
				-+-----+-----+-----+-----
Pooled StDev = 1.620				56.0 58.0 60.0 62.0

Fisher's pair wise comparisons

Family error rate = 0.176

Individual error rate = 0.0500

Critical Value = 2.306

Appendix 6: ANOVA ANALYSIS (One-Way ANOVA: C2 versus C1) for head weights

One-Way ANOVA : C2 versus C1

Analysis of Variance for C2

Source	DF	SS	MS	F	P
C1	3	0.70	0.23	0.23	0.875
Error	8	8.25	1.03		
Total	11	8.95			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	
1	3	3.665	1.686	(-----*-----)
2	3	3.905	0.419	(-----*-----)
3	3	3.583	0.573	(-----*-----)
4	3	3.230	0.882	(-----*-----)

Pooled StDev = 1.015

Fisher' pairwise comparisons

2.0 3.0 4.0 5.0

Family error rate = 0.176

Individual error rate = 0.0500

Critical value = 2.306