INCIDENCE, RACE DETERMINATION AND SURVIVAL OF BLACK ROT PATHOGEN (*Xanthomonas Campestris* pv *campestris)* IN *Brassica* SEEDS AND CROP DEBRIS IN KENYA

By

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DECLARATION

Declaration by the Candidate

I declare that this thesis is my original work and it has not been presented in this or any other University for any degree. No part of this thesis may be reproduced without prior permission of the author and/or University of Eldoret.

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DEDICATION

This thesis is dedicated to my family John Lang'at, my sons Bernard Koech, Richard Koech and Evans Koech for their tireless effort, contributions, patience, encouragement, incredible hard work and discipline while pursuing Master of Science Studies. I want to assure them that, this is the beginning of better things a head. Cheers everyone.

ABSTRACT

Xanthomonas Campestris pv *campestris* (Xcc) is a seedborn pathogen that cause black rot, a destructive disease of *Brassica* and the primary source of inoculum. Xcc. is the major problem of infestation in *Brassica* seeds. Therefore imported and locally available seeds for production in Kenya were investigated for the presence of black rot and survival in *Brassica* debris. The objective of the research was to assess the incidence, survival and races of the pathogen in seed *Brassica* stock which are currently used for production in Kenya and survival of black rot in *brasssica* debris and race typing of the pathogen. The races found to be pathogenic to susceptible savoy cabbage Wirosa F1 were race type 1 and 3. The incidence and survival of black rot was conducted concurrently during the study at Kenya Plant Health Inspectorate Service Laboratories and National Agricultural Research Laboratories, Nairobi. The results show that Xcc is widespread in *Brasssica* seeds and survive in the *Brasssica* debris three months. Samples of cabbage, kale and collards seed were purchased from a variety of sources and locations in Kenya. Xcc infested cabbage and kale crop debris were collected from a smallholder's farm and initially tested for the presence of the pathogen. The remains of the infested debris were left in the fields as farmers leftovers. They were collected at random for testing fortnightly for the presence of black rot for 6 months. The Xcc detected varied with respect to the types of Brassica, cultivar and seed source. The kales had the highest number of Xcc, followed by collards and cabbages. The type of *Brassica sp* (χ^2 = (28.66), (5), cultivar (χ^2 = (36.73), (17) and *seed* source (χ^2 = (30.46), (11) influenced the Xcc detected. The Xcc detected was not influenced by collection region and origin of *Brassica sp.* The number of Xcc present in kale was slightly more than those found in cabbages. However there was no statistically significant difference on the number of Xcc present. The number of Xcc present in the debris depends on the existence of debris and the survival of bacteria decreases with increase in duration of exposure. The survival and existence of Xcc present in the soil was significant with time. The Xcc in the soil reduced with the length of time it is exposed. The Xcc race type 1 and 3 were obtained. The recommendations for the management of black rot is leaving the field without any *brasssica* or alternate hosts for about eight months and using certified seeds for multiplication.

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LIST OF ABBREVIATIONS

Xcc	*Xanthomonas Campestris* pv *campestris*
ISTA	International seed testing association
YDC	Yeast Dextrose Chalk-Agar medium
FS	Agar medium-Selective media for *Xanthomonas Campestris* pv *campestris* in crop debris.
m CS20 ABN	Agar medium- Selective media for *Xanthomonas Campestris* pv *campestris* in crop debris.
CCP	Critical Control Points for *Xanthomonas Campestris* pv *campestris* in crop debris.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Plants may be affected with black rot at any stage of growth. Seedling infection first appears as a blacking along the margin of the cotyledon (seed leaves), which later shrivels and drops off. Affected seedlings turn yellow to brown, wilt, and collapse. Leaf infections often result in a small, wilted, V-shaped infected area that extends inward from the leaf edge toward the midrib. Diseased areas enlarge and progress toward the base of the leaf, turn yellow to brown, and dry out. The veins of infected leaves, stems, and roots turn black as the pathogen infest. Black rot of crucifers is characterized by blackened vascular tissues and foliar marginal V-shaped chlorotic or necrotic lesions (Cook *et al.*, 1952a).

Black rot is a bacterial disease of *Brassica* that affects cabbage and related crops like *Brassica,* mustard and radish worldwide and is caused by the bacterium *Xanthomonas Campestris* pv *campestris (Xcc).* It is one of the most serious diseases of cabbage and kales in warm climates.Black rot bacterium can over-season in infected cabbage seeds and weeds belonging to the *Brassicaceae* family (including black mustard, field mustard, wild turnip, wild radish, shepherd's purse and pepper weed); or in infested plant debris in the soil. A very destructive disease often starts with infected seeds as the initial inoculum even though infected seeds frequently appear healthy. There are strong indications that disease outbreaks are mainly caused by internal seed infections, hence, the use of seed

free from infections is one of the important ways to avoid disease (Kohl and Van der Wolf 2005).

Incidence of black rot is well recognized worldwide. Periodical epidemics of the disease were usually ascribed to the introduction of susceptible cultivars, use of infected seeds and seedlings and weather conditions that favor disease development. *Xcc* is always found in association with infected plants. Strains that are known to be pathogenic are differentiated into 123 pathovars, pathogenic variants (Vicente *et al.*, 2002). On the basis of limited pathogenicity tests, some members of a pathovar may have a broader host range than others since exhaustive pathogenicity testing is impractical. Other than these tests, there is no definitive means of classifying an unknown *Xanthomonas Campestris* pv *campestris* isolates.

Strains that are not pathogenic are unclassifiable by the pathovar system, yet they may exhibit similar host range specificity to those which are pathogenic. This pathogen can infect a wide range of plants within crucifer, including cabbage, cauliflower, kale, rape, radish and black mustard. The pathogen once established repeatedly multiplies and spread when conditions are favourable. Other primary sources of infection can be soil and splashes dispersed from adjacent infected fields, infected perennial weeds, infected machines and materials and possibly insects. Soil is a well-known source of inoculum.

Xanthomonas Campestris pv *campestris* can only survive for 20-50 days in soil, and two years in cabbage residues in soil (Schaad and White 1974; Dzhalilov and Tiwani, 1995;

Kocks *et al.,* 1986). While, Bombay and Murkherjee, (1955) reported the severe incidence of black vein (symptoms of black rot disease) of cabbage in West Bengal caused by *Xanthomonas Campestris* pv *campestris.* Rao and Srivastava (1964) reported a widespread occurrence of black rot of cabbage in India. The *Brassica* seeds commonly grown in Kenya are imported from Europe, United States of America (U.S.A) or South Africa (African Farming, May/June 2005). All popular varieties grown by smallholder farmers in Kenya are at least 20-40 or more years old, bred outside Kenya and are not adapted to the prevailing conditions, for example disease resistance.

Seed treatments like bulk of chemical seed treatments have been targeted at fungal pathogens leaving the option of using clean seed and disease free seedbeds in terms of *Xanthomonas Campestris pv campestris* pathogen. A range of physical treatments (hot water, hot hair, electron bombardment, for control of bacterial diseases of *Brassicas* caused by *Xanthomonas Campestris* pv campestris reduced seed infestation levels and did not eliminate the pathogens (Robert *et al.,* 2006). From the 11th international conference on plant pathogenic bacteria, Edinburgh, 10th-14th July 2006 there is need therefore to use only clean seed in disease free farm to produce clean seed lot.

1.2 Statement of the Problem

Black rot disease is one of the major problems that constraints potential production of *Brassica* vegetables in Kenya. It would be difficult to develop a control strategy based on the use of disease free seed, without evidence that the clean seed could potentially be infected by *Xanthomonas Campestris* pv *campestris* present in *Brassica* crop debris or contaminated seed bed. The disease is spread by seed, seedlings and insects. As few as 3

infected seeds in 10,000 can seriously infest an entire cabbage field (Robert *et al.*, 2003). The pathogen survives on crop residues and related *Brassica* weeds for several years after a field is infested thus the economic and nutritional burden imposed by the pathogen endures longer than a single cropping season. No seed treatment reliably eliminates *Xcc* (Fatmi *et al.*, 1991).

At a recent meeting (review of the Crop Protection Programme) which concentrated in Peri Urban African Vegetables October 2002,) black rot was considered as a serious threat to *Brassica* seed production and was highlighted as a future research priority. In addition to seed, an additional potential source of primary inoculum in intensively cropped *Brassica* production areas is the infected crop residues (debris) which remain in the fields after harvesting. Therefore this research seeks to examine the incidence of *Xcc* in *Brassica* seeds used, imported, seeds locally produced in Kenya, race type and the presence and survival of *Xanthomonas Campestris* pv *campestris* in *Brassica* crop debris.

1.3 Justification

Kenya's economy is based on agriculture. *Brassica* vegetables are the key commodities within the agro-industrial sector and also provide nutrition, income and employment to people of Kenya. However *Brassicas* are grown by both small and large scale farmers using formal and informal seeds. Production of *Brassica* is constrained by black rot pathogen caused by *Xanthomonas Campestris* pv *campestris. It* is a threat to *Brassica* production in Kenya especially for the domestic market as it survives in seeds, *Brassica* debris and even in soil, making it increasingly difficult to manage by Kenyan farmers. Therefore it is important to examine the health status of the seeds available for production and also the survival of the pathogen in crop debris.

Seed health is one of most important aspect of seed quality as this could improve both livelihoods of producers and nutrition of consumers. An important advantage of using clean seed is that it reduces use of chemicals. Chemicals pose a risk to the consumers because of chemical residues in produce and environment. Furthermore seeds treated with chemicals have no effect on the bacteria pathogen. The only remedy therefore, is to use disease free seeds. Lack of improved/resistant varieties is another major constraint, which has led to yields amounting to about one-quarter of those achieved in top-producing countries (AF, 2005).

The annual production is estimated to be over 1.5 million tonnes of vegetables on an estimated 100,000 ha, with 90-95 % being consumed domestically and just 5-10% exported. Most production (70-80% of marketable product) is produced by both small and large scale farmers (AF, 2005). Black rot disease is endemic and causes much damage in Kenya (Onsando, 1992). It is considered that improving seed quality and seed systems could considerably improve both the livelihoods of producers and also the nutrition of the consumers. This research sought to determine the presence and survival of *Xanthomonas Campestris* pv *campestris* in *Brassica* crop debris and seeds stock obtained from a range of sources that are currently used for production in Kenya.

1.4 Overall Objective of the Study

To assess the incidence of black rot pathogen in *Brassica* seeds which are used for production in Kenya and to evaluate the presence and survival of *Xanthomonas*

Campestris pv *campestris* in crop debris and the race identification of *Xanthomonas Campestris* pv *campestris* found in Kenya.

1.5 Specific Objective

1. To assess the incidence of *Xanthomonas Campestris* pv *campestris* in *Brassica* seeds available for production in Kenya.

2. To determine the presence and survival of *Xanthomonas Campestris* pv *campestris* in *Brassica* debris in Kenya.

3. To determine the races of *Xanthomonas Campestris* pv *campestris* found in Kenya.

1.6 Hypothesis

Ho1: There is no incidence of *Xanthomonas Campestris pv campestris* in *Brassica* seeds available in Kenya

H_o2: There is no presence and survival of *Xanthomonas Campestris* pv *campestris* in *Brassica* debris in Kenya.

H_o3: There are no races of *Xanthomonas Campestris* pv *campestris* found in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Production of Different *Brassicas*

Brassicas occupy the largest area of cultivated crops, with about 250,000 ha grown annually (reference). They include species and inter-specific hybrids crops that produce primarily root biomass for example, swede (*Brassica napus L*.) and turnip (B. rapa L.), others that produce mainly leaf and stem for instance kale (*B. oleracea L*.) and rape (*B. napus L*.) and some that produce much leaf but little stem or root for example Pasja, a fast-growing, early maturing turnip, chinese cabbage hybrid and cabbage. Brassica belong to the family Brassiceae and are economically categorized into oilseed, vegetable and condiment crops.

Brassica napus, *Brassica* rapa (formerly campestris), *Brassica juncea*, and *Brassica carinata* provide about (12% of the reference) worldwide edible vegetable oil supplies. B. oleracea and Brassica rapa, the so-called cole crops comprise many of the vegetables in our daily diet. Several of these vegetables have extreme morphological characteristics. Examples of such morphologies include the enlarged inflorescence of cauliflower (*B. oleracea* subspecies botrytis) and broccoli (*B. oleracea* subspecies italica), the enlarged stem of kohlrabi (*B. oleracea* subspecies *gongylodes*) and marrowstem kale (*B. oleracea* subspecies *medullosa*); the enlarged root of turnip (*B. rapa* subspecies *rapifera*), the enlarged and twisted leaves of Pak-choi (*B. rapa* subspecies *chinesis*) and Chinese cabbage (*B. rapa* subspecies *pekinesis*); and the enlarged single apical bud of cabbage (*B. oleracea* subspecie*s capitata*) or the many axillary buds of Brussels sprout (*B. oleracea*

subspecies gemmifera and the seed of *B. nigra* is utilized as a condiment. Brassica species are a valuable source of dietary fiber, vitamin C and other likely salubrious factors such as anticancer compounds (Hough *et al.*, 2000).

Vegetable production provides nutrition, income and employment to more than 4 million poor people in Kenya. *Brassicas* (kale and cabbage) are the most important vegetables for the domestic economy, being grown by more than 90% of smallholders. However, vegetable productivity is constrained by both the availability and quality of seed, pest and diseases. Much of the seed that is currently available is expensive, imported seed, and the quality is poor. There is a need of raising awareness of the importance of using good quality seed and establishing a commercially (Spence *et al.*, 2005). Estimates of the economic importance of *Brassica* species are several cole crops such as collards which are cultivated primarily for local or home use, but are dietary mainstay in low-income communities where other fresh vegetables can be expensive. Black rot is characterized by blackened vascular tissues and follows marginal v-shaped chlorotic or necrotic lesions (Cook *et al.*, 1952a). As the disease progresses, parenchyma cells surrounding vessels in the main stem turn black, and the plant becomes wilted, stunted and finally rots.

2.2 Geographical Distribution of Black Rot

The pathogen that causes black rot is widely distributed in Africa, Asia, Australia and Oceania, Europe, North America, Central America, the West Indies and South America (CABI, 2005). Black rot is endemic in Africa. It is the most important disease of *Brassicas* in Kenya, Zimbabwe and Zambia (CABI, 2005).

Source: Infonet-biovision.org

Figure 1: Geographical Distribution of black rot in Africa red marked

2.3 Damage caused by Black Rot

In Kenya, black rot is endemic and the cause of much damage (Onsando *et al.,* 1988, 1992). The disease is considered of intermediate economic importance in Mozambique (Plumb-Dhindsa and Mondjane, 1984). Black rot is widespread in Zimbabwe where it is considered the most important disease of *Brassicas* (Mguni, 1987, 1995).

2.4 Host Range of Black Rot Pathogen

Since the early 1990s, diseases caused by black rot have been spreading on new host plants and in new regions that had not been previously affected by the pathogen. Still vegetable crops of *Brassica oleracea* are the most damaged plants by black rot Cauliflower, cabbage and kales are the most readily affected hosts in the crucifers but broccoli and Brussels sprouts have intermediate resistance to black rot.

2.5 Symptoms of Black Rot on Cabbage

The plant can be infected at any stage during its life cycle. On young seedlings a yellowing appears along the margin of the cotyledons, which later shrivels and drops off. On the margins of mature leaves, similar yellowing appears. Initially a small V-shaped area develops but as the diseased area enlarges, the veins become distinctly black. The affected plant stages include; seedlings, vegetative growing stage and heading stage (cabbages). The plant parts affected comprise leaves, seeds, stems, vegetative organs and the whole plant. Affected plant parts are evident by different symptoms for example leaves portray V-shaped lesions, seeds show discolorations lesions, stems and vegetative organs have internal discoloration which are black in colour and leads to the death of the whole plant.

2.6 Biological and Ecology of Black Rot

The black rot disease survives in infected seed, debris from diseased plants left in the field and in infested soil. Seed-borne bacteria can be disseminated long distances; many cruciferous weeds can harbor the black rot bacteria.

Plate A: Black rot damage

Plate B: Black rot on the young cabbage

Plate C: Bacterial black rot on the leaf of cabbage

Plate D: Black rot on cabbage

Plate E: Black rot on stem

Plate F: Bacterial black rot on kales

Plate G: Initial infestation of black rot on cabbage

Source: Infonet-biovision.org

Figure 2: Various symptoms and Ecology of Black Rot

In a new field, black rot is via infected seed or diseased transplants. The bacteria enter the plant mainly through water pores at the edges of leaves. They can also enter through the root system and wounds made by chewing insects. They then move through the water vessels to the stem and head. Black rot is favoured by warm (26^0-30^0 c) wet conditions. Characteristic yellowish V-shaped areas at the leaf margin, sites of infection by *black rot.* Blackening of water-conducting tissue is of the stem as a result of black rot infestation.

2.7 Survival of Xcc in the Absence of the Host

Carry-over of inoculum of *Xanthomonas Campestris* pv c*ampestris* was investigated in the soil from one cropping season to the next in field experiments over three years (Kocks *et al.,* 1998). These studies were supported by laboratory and greenhouse experiments on quantitative assessment of bacteria by bioassay using the most probable number technique, and on recovery rates of bacteria from the soil. The mean recovery rate from artificially infested soil was 58%. Extinction of *Xanthomonas Campestris* pv c*ampestris* in soil infested with infected plant debris proceeded exponentially and extinction rates depended on temperature, as did the decomposition of plant debris. In replicated field plots, over three years, infection foci of black rot disease were established.

At harvest time, all plants were chopped and resulting plant debris was rotovated into the soil. The resulting soil infestation was sampled and showed clear infestation reflecting the original infection of the crop. This infestation decreased with time and disappeared after the winter. Follow-up crops remained virtually uninfected. The results show that in The Netherlands good crop and soil management impedes survival of inoculum from one

year to the next, so that cabbage can be grown continuously. Polyetic carry-over of inoculum by debris in the soil can be avoided in the Netherlands (Zhao *et al.,* 1999; 2000). Detection, Survival, and Sources of *Xanthomonas Campestris* pv c*ampestris* of leafy crucifers in Oklahoma. *Xanthomonas Campestris* pv. *campestris, Xanthomonas Campestris* pv. *armoraciae*, and *Pseudomonas syringae* pv. *maculicola* are bacterial pathogens that cause leaf spot diseases on leafy crucifers in Oklahoma.

A simple, rapid PCR method based on primers from the gene was developed to detect coronatine-producing strains of *Pseudomona syringae* in plants. Pathogenicity tests confirmed the positive strains to be *Pseudomonas syringae* pv. *maculicola*. To monitor the survival of *Xanthomonas Campestris* pv. *armoraciae* and *Pseudomonas. syringae* pv. *maculicola* in the field, turnip and collards were inoculated with rifampicin-resistant strains and were buried beneath the soil or left on the soil surface. Both pathogens were recovered from turnip and collard debris up to 2 months following burial, but neither pathogen was recovered from soil after the debris had decomposed. However, both pathogens were recovered from plant debris left on the soil surface for up to 5 months.

Four production fields were surveyed for sources of inoculum of the bacterial pathogens from October 1999 to May 2000. *Xanthomonas Campestris* pv. *campestris* was isolated from the weed shepherd's purse (*Capsella bursa-pastoris*) in all fields, and from volunteer turnip and kale in three fields. *X. campestris* pv. *campestris* and *Pseudomonas syringae* pv *maculicola* were isolated from surface debris and regrowth from crop stubble left in one field after harvest in the fall. *Xanthomonas Campestris* pv. *campestris* was

detected in 6 of 51 lots of crucifer seed assayed. *Xanthomonas Campestris* pv. *armoraciae* and *Pseudomonas syringae* pv. *maculicola* were not recovered from weeds, volunteer plants, or seed lots.

2.8 Strains of *Xanthomonas Campestris* pv *campestris*

According to the work done by Raymond *et al.,* (1987) *Xanthomonas Campestris pv campestris* is always found in association with plants. Those *Xanthomonas Campestris* pv *campestris* which are known to be pathogenic are differentiated into 123 pathovars (pathogenic variants), on the basis of limited pathogenicity tests. Some members of a pathovar may have a broader host range than others, since exhaustive pathogenicity testing is not practical. Other than these tests, there is no definitive means of classifying an unknown *Xanthomonas Campestris* pv *campestris* isolate. Those *Xanthomonas Campestris* pv *campestris* which are not pathogenic are unclassifiable by the pathovar system, yet they may exhibit similar host range specificity to those which are pathogenic.

2.9 Races of *Xanthomonas Campestris* pv campestris

The races, identification, origin and related of *Xanthomonas Campestris* pv. *campestris* were studied and classified as one hundred sixty-four isolates of *Xanthomonas Campestris* pv. *campestris* and other *X. campestris* pathovars known to infect cruciferous hosts (*X. campestris* pvs. *aberrans*, *raphani*, *armoraciae*, and *incanae*) were inoculated onto a differential series of *Brassica* spp. to determine both pathogenicity to brassicas and race. Of these, 144 isolates were identified as *X. campestris* pv. *campestris* and grouped into six races, with races 1 (62%) and 4 (32%) being predominant. Other races were rare. The remaining 20 isolates from *Brassicas* and other cruciferous hosts were either

nonpathogenic or very weakly pathogenic on the differential series and could not be race-typed (Vicente *et al.,* 2001).

2.9.1 Biological Control

In experiments to investigate the antagonism of eight *Bacillus* isolates against nine strains of *Xanthomonas Campestris* pv. *campestris* to assess the role of lipopeptides in this process. Antimicrobial and hemolytic (surfactant) activity tests were performed in-vitro using agar diffusion methods. Antibiosis and hemolysis were positive for four *Bacillus* isolates against all *Xanthomonas Campestris* pv.*campestris* strains Monteirol *et al., (*2005).

2.9.2 Physical Control

Physical sanitation methods are used by the seed industry to prevent transmission of seed-borne diseases, but sensitivity varies between seedlots. The effect of seed maturity on the sensitivity to hot water, aerated steam and electron treatments was studied. Two *Brassica oleracea* L. and two *Daucus carota* L. seed lots from commercial production were selected for containing relatively large amounts of less mature seeds. Each seed lot was sorted into three maturity fractions based on the levels of chlorophyll fluorescence of individual seeds. Less mature *B. oleracea* and *D. carota* seeds were more susceptible to hot water treatments and less mature *B. oleracea* seeds to the aerated steam treatment. Seed maturity did not influence the sensitivity to the applied electron seed treatments. Seed lots were not selected for infections with seed-borne pathogens, however the less mature seeds were observed to be more frequently infected Groot *et al.,* (2005/2008).

Incidence of *Xanthomonas Campestris* pv *carotae* on carrot seed irrigated by drip or sprinkler in central oregon, (Crowe *et al.,* 2004) may be infested with 106 to 108 colony forming units (CFUs) of Xcc per 10,000 seeds (du Toit *et al.,* 2005), which requires hot water treatment Pscheidt and Ocamb (2001) to lower the infestation to acceptable levels that would not likely develop into a bacterial problem in commercial fields. In fact, hot water treatment generally eliminates detectable *Xanthomonas Campestris* pv. *campestris* (Robert *et al.,* 1999) transmission from seed to seedling and secondary spread of *Xanthomonas Campestris* pv. *campestris* from seeds to seedlings of cauliflower were investigated. Seeds inoculated with different concentration of bacteria were subjected in four different watering regimes for example high frequency, overhead spray, low frequency spray, high frequency capillary and low frequency capillary and the effects of treatments on symptoms and on the proportion of contaminated were found to be similar.

2.9.3 Cultural Control

Use of certified disease free seeds, establishing crops in seedbeds free of black rot, free soils that have not grown crops from crucifers family. The seedlings ought not to be crowded in the nursery. Irrigation with contaminated water should be avoided, rremoval of crop debris after harvest, control of cruciferous weeds that may serve as reservoir for the pathogen and sanitation for example using clean equipment's, avoiding work in wet fields.

2.9.4 Chemical Control

The chemical control of black rot using fungicides has not been effective however the spread of black rot in the fields may be slowed through applications of fixed coppers.

2.9.5 Seed Treatment

When cauliflower plants (*Brassica oleraceae*) were misted with bacterial suspensions of *Xanthomonas Campestris* pv. *campestris*, two separate populations of the pathogen were associated with the leaves. Bacteria removable by sonication and sensitive to sodium hypochlorite treatment predominated however after 2 weeks; bacteria not removable by sonication and insensitive to sodium hypochlorite treatment were dominant. These results indicate that a functional *pig B* is required for epiphytic survival and natural host infection under the experimental conditions tested, and could all be important for survival of this pathogen on the leaf surface, and/or for host infection (Poplawsky and Chun 1989).

2.9.6 Integrated Pest Management (IPM)

Integrated pest management is an approach to pest control that considers all management options to maintain pests below an economic injury level. Tools for the management of pests include cultural, physical, biological, behavioral and chemical. With IPM, adverse effects of pesticides are minimized and economic returns are maintained. Use of certified seeds, clean seeds or free plant materials is an important and potentially high effective means of disease control for seed-borne diseases.

CHAPTER THREE

MATERIALS AND METHODS

3.1 *Brassica* Seed Collection

Data for this study was derived from various sources for example seed merchants, stockists, small scale farmers, local markets and hawkers. Samples of cabbage, kale and collards seeds were purchased from a variety of sources and locations throughout the country-Kenya. Seeds were collected from August 2007 to May 2008 with locations ranging from Mombasa in the East to the Ugandan border in the West. The areas included the former provinces; Coast, Eastern, Central, Nairobi, Rift Valley, Western and Nyanza province. Most seeds were obtained in sealed tins of various sizes for example, sachets, 50gm and 100gm of various sizes depending on the availability of the seed lot. Each sample was allocated a unique identification number and the details recorded during analysis process. Where there was sufficient seeds, samples were divided into sub-samples for testing.

3.2 Extraction Methods

The extraction methods for *Xanthomonas Campestris pv campestris* (Black rot) was adopted. The Brassica seeds for extraction of Xcc were subjected to series of seed tests of which the maximum sub sample size should be 10,000 seeds that formed the working sample.

3.3 Test Method and Identification

The seed test method used was described by (Roberts *et al.* (2003), and the recommended centrifugation step (Appendix 1). Briefly the steps were as follows:

3.3.1 Preparation of Seeds for extraction of Xcc

Each seed lots of 30,000 seeds were allocated unique sequential sample No. i.e. 782 and details recorded i.e. source, lot no TSW, seed treatments and each sub sample of a lot is also given a No. i.e. 782/1, meaning seed lot No. 782 sub sample 1. Then only one 782/1 regardless of the test dates. Each colony sub-cultured from a sub sample were also given a number which was unique within the sub-sample, regardless of dilution e.g. 782/1/1 means lot 782, sub-sample 1, colony 1, colonies 1-3 could come from one dilution and colonies 4-6 could come from another dilution records where they came from by writing the colony no's on the dilution plates.

Ten thousand (10,000) seeds were suspended in extraction sterile 0.85% saline plus 0.02% Tween 20 (approximately 10 ml per 1,000 seeds) in conical flasks and shaken for 2.5 hours on an orbital shaker. Two serial ten-fold dilutions were prepared and the cultures from the seed extract were spread into plates containing selective medias of FS and mCS20ABN. Cultures were incubated at 28- 30°C for 3-4 days after which the plates were examined for the presence of typical Xcc colonies.

3.3.2 Details of Xcc extraction and Preparation of Seeds

Extraction of Xcc was prepared as follows:

Seeds were suspend in pre-chilled (2-4 degrees centigrade) sterile saline plus tween 20 (0.02%) in a conical flask. The volume of saline should be adjusted according to the No. of seeds used (10ml per 1,000 seeds). It was then shaken for 5 min at room temperature (20-25 degrees centigrade) on an orbital shaker set at 100-150 rpm. A pipette of two 1 ml samples of the seed extract was put into two separate sterile micro-centrifuge tubes and

kept on ice or in the refrigerator until processed. This it was shaken continuously in the flask at 100-150 rpm at room temperature for 2.5hours.

Centrifugation

The centrifugal procedure was done using the following steps;

A centrifuge of two 1 ml samples of extract was done at 11,600 for 10 minutes. A 0.8 to 0.9 ml was removed from each of the micro-tubes taking care not to disturb the pellets. The vortex was re-suspended to the pellets in the remaining liquid. It was kept on ice or in the refrigerator until plated. This was pipetted on the entire volume (100-200 ul) of each of the re-suspended pellets into plates of each of the selective media (FS, mCS20 ABN) and spread over the surface with a sterile bent glass rod. Then the incubate plates was used. The flasks were shaken to be mixed just before dilution. This prepared two serial ten-fold dilutions from the seed extract. Pipette of 0.5ml of the extract was made into 4.5ml of sterile saline and vortex to mix (10 dilutions). Pipette of 0.5ml from the 10 dilutions were made into another 4.5ml of sterile saline and vortex to be mixed (10^2 dilutions). A pipette of 100 ul of each dilution and the undiluted seed extract was made into plates of each of the selective media (FS, mCS20 ABN) and spread over the surface with a sterile bent glass rod. This was incubated in plates at 28-30^0c and examined after 3-4 days.

Positive Control (Culture or reference material) was achieved through preparing a suspension of a known strain of Xcc in sterile saline. This was diluted sufficiently to obtain dilutions containing estimated, 10^2 to 10^4 cfu/ml. This prepares 100 ul of appropriate dilutions into plates of each of the selective media (FS, mCS 20 ABN) and

spread over the surface with a sterile bent glass rod. Incubate plates with the sample plates. Sterility check was made by preparing a dilution series from a sample of the extraction medium i.e. saline + tween 20 containing no seeds and plate on each of the media as for samples.

Examination of the plates was done through **e**xamining sterility check and positive control plates (CCP). The sample plates were examined for the presence of typical Xcc colonies by comparison with positive control plates. After 3-4 days, the FS and Xcc colonies were small, pale green, mucoid and surrounded by a zone of starch hydrolysis. This zone appeared as a halo that may be easier to see with a black background. Colonies showed variation in size and may be visible on Fs after 3 days. After 3-4 days on FS, mCS 20 ABN, Xcc colonies are pale yellow, mucoid and surrounded by a zone of starch hydrolysis, colonies may show marked variation as in size as well. The records on the number of suspects were done.

3.3.3. Examination of the Cultures for the presence of Xcc Colonies-

After 3-4 days, Xcc colonies on selective media FS and mCS 20 ABN, were examined and results recorded.

Confirmation / identification of suspect colonies: Suspect colonies of Xcc were Sub-cultured to sectored plates of yeast dextrose calcium carbonate (YDC) agar medium. The positive control isolate were Sub-cultured to a sectioned plate for companion. The sectioned plates were incubated for 24 – 48 hours at 28-30 degree centigrade. The appearance of growth was compared with positive control. The positive colonies were harvested for pathogenicity and race determination.

3.4 Pathogenicity

The identity of isolates was confirmed by pathogencity on *Brassica* seedlings of known susceptibility, for example, cabbage CV *wirosa*. The seedlings of cultivar *wirosa* were grown in small pots until 2 – 4 leaf stage. Small amount of bacterial was inoculated with sterile insect pin on the two youngest leaves on the major veins. Plants were examined for the appearance of typical progressive V-shape, yellow necrotic lesions with blackened veins after 7- 14 days of inoculation

3.5 Determination of Races of Xcc

Bacterial growth was scraped from the plates, three leaves were picked the youngest and two others were visually selected and inoculated with Xcc by pricking on the secondary veins near the margins with sterile pins dipped into the concentrated 100% bacteria isolate (Vicental *et al;* 2003). Three to four points of inoculation were made per leaf, and the three youngest leaves on each plant were inoculated. Plants were observed for the appearance of typical black rot symptom after three to four weeks of inoculations. The number of infected points per leaf and the severity of symptoms were assessed and were rated on a scale of 0 to 4 (Kamoun's *et al;* 1990).

Six races of *Xanthomonas Campestris* pv. *campestris* were identified on the basis of their reactions on a series of differential *Brassica* genotypes. Races 1 and 4 are the most important races worldwide. The positive Xcc colonies were harvested for pathogenicity and race determined the reaction of the differential cultivars described by (Kamoun *et al*., 1990) was used for the initial separation of *Xanthomonas. campestris* pv. *campestris* isolates into races. Isolates corresponding to Kamoun's races 0, 1, 2, 3 and 4 were used

and also isolates showing the pattern of reaction of Kamoun's race 0, 2 and 4 (Table 1 below. The number of infected points per leaf and the severity of symptoms were assessed and rated on a scale of 0 to 4 (Kamoun's *et al.,* 1990) based on the relative size of the largest lesion on the leaf: 0, no symptoms; 1, slight necrosis or chlorosis surrounding the infection point; 2, typical V-shaped yellow or necrotic lesion with blackened veins with a lesion size of less than 1 cm, 2, and 3, typical V-shaped lesion with a size of more than 1 cm^2.

Table 1: List of *Brassica* accessions used in Pathogenecity test to identify *Xanthomonas Campestris* pv *campestris* races

Differential cultivars	**Species**	**Interaction with race/resistance**						
		1	2	3	4	5	6	7
Wirosa F1	*Brassica oleracea*	+	+	+	+	+	+	+
Just Right Hybrid Turnip	*Brassicarapa*	+	+	+	-	+	+	+
Cob60	*Brassica napus*	+	+	+	-	+	+	+
Seven Top Turnip	*Brassica rapa*	+	-	+	-/+	-/+	+	+
Bo99075 (PI 199947)	*Brassica corinata*	-	+	-	-	+	+	+
FBLM2	*Brassica juncea*	-	+	-	-	-/(+)	+	-
Miracle F1	*Brassica oleracea*	+	-	-	+	-	(+)/+	+
SxD1	*Brassica oleracea*	+	-	-	+	-	(+)/+	+

+ Compatible interaction (susceptible host); |- incompatible interaction (resistance host); (+) weakly pathogenic; -/+ variable results within accession; Source Vicentre *et al.* (2001).

3.6 Determination of the Presence and Survival of Xcc in *Brassica* Crop Debris

In addition to seed, another potential source of primary inoculum in intensively-cropped *Brassica* production areas is the crop residues (debris) which remain in the fields after

harvesting of an infected *Brassica* crop. Thus in order to gain an understanding of the importance of crop debris as a source of primary inoculum in seed production, an attempt was undertaken to examine the survival of the pathogen in crop debris or in the soil over a period of six months from January to July 2008. In order to determine the presence of *Xcc*, infested cabbage and kale crop debris was collected from a smallholder's farm at Thika and tested for the presence of Xcc before subjecting into the plots to confirm the presence of the pathogen and after tests, they were found to be positive.

3.6.1 Trial /layout

A summary of the treatments is shown in Table 2. The trial consisted of five plots. Each plot was approximately. 3 m x 2 m and was separated from the other plots by at least 10m. Cabbage debris was applied to two plots and kale debris was applied to two plots, a mixture of cabbage and kale debris was applied to the fifth plot. In one cabbage plot and one kale plot, the debris was applied to the surface incorporated into the soil by hoeing. The remaining plots were covered with nets to prevent the debris on the surface from blowing away. The debris was just left the way the farmer harvest and leaves the debris on the surface or ploughs them into the soil.

Table 2: Treatments to assess the Survival of *Xanthomonas Campestris* pv. *campestris* in crop debris.

Plot	Debris	Weight	Treatment
A	Cabbage	950 g	Debris incorporated into the soil
B	Cabbage	950 g	Debris spread on the surface and covered with netting
C	Kale	300 g	Debris spread on the surface and covered with netting
D	Kale	300 g	Debris incorporated into the soil
E	Cabbage + kale	850 g	Debris spread on the surface and covered with netting

Sampling and testing started from January to August 2008

Samples of the debris and/or soil were collected fortnightly from each plot for six months following initial set up and continuing until *Xcc* was not detected on three consecutive occasions. The presence of *Xcc* in the samples was determined using a protocol devised by Roberts *et al.* (2003), using selective media (FS and mCS20 ABN) as used for seed health testing. Debris/soil samples were suspended in sterile saline plus Tween 20 (0.85% NaCl, 0.02% Tween 20; 10 ml per gram of debris/soil) in conical flasks. Samples were then allowed to soak for 1.5 h and shaken on an orbital shaker to mix. Four serial ten-fold dilutions were prepared, and 0.1 ml of each dilution and the un-diluted extract were spread onto the surface of plates of FS and mCS20ABN semi-selective media. Plates were incubated at 30°C for 3-4 days and the suspect *Xcc* colonies were recorded. The identity of suspect *Xcc* colonies was confirmed in the tests.

3.7 Data Analysis

Statistical measures were generated using Statistical Packages for Social science (SSPS) Version 20. This statistical package was chosen because it provided comprehensive

statistical capabilities as well as features that make easier to access and manage data; select and perform analysis. Data collected were analysed using both descriptive statistics; means, standard deviation, and standard error-and inferential statistics including Analysis of Variance (ANOVA), and Cross tabulation Chi-square. Use of descriptive statistics for the variables under study was preferred method as this method was adopted to describe the demographic characteristics and also to explain the incidences of Xcc and the differences that exist among the variables of the study. The Analysis of Variance was used in occasions where the data had more than one dependent continuous variable or more than two values across the categorical independent variables, used to compare the Xcc variation in *Brassica* sp.

CHAPTER FOUR

RESULTS

4.1 Incidence of Xcc in Brassica seeds obtained from various sources in Kenya.

The first objective was to assess the incidence of *Xanthomonas Campestris* pv *campestris* in *Brassica* seeds available for production in Kenya. This was achieved by establishing the incidence of Xcc in *Brassica* seeds with respect to the type, cultivar, supplier, origin, sample size and Xcc detected. This objective summarizes the results of the tests of the seeds collected throughout the country for the purposes of detection of *Xanthomonas Campestris pv campestris* in the seed lots available for farmers in Kenya.

The results include the country of origin of the seeds and the type of *Brassica* that tested positive. Both imported and locally available seeds tested positive when only a sub sample was tested, which means that, had we tested the second sub sample and more, we would have expected more positives. These results then justify the first objective of the study that the occurrence of *Xanthomonas Campestris* pv *campestris* in *Brassica* seeds available for production in Kenya. Therefore incidence of Xcc is vital in this country both for imported and locally available seeds. All this were determined using descriptive and inferential statistics. The frequency and percent represented the descriptive statistics whereas the cross-tabulation and analysis of variance were inferential statistics.

4.1.1 Type of *Brassica* seeds and incidence of Xcc

During the study five types of *Brassica* seeds were involved as summarized in Table 3. Out of 110 incidences investigated, cabbages comprised of 50(45.5%) of the *Brassica*

seeds, 26 (23.6%) collards, 30 (27.3%) kale, 1.8% Chinese cabbage, and 1 (0.9%) cauliflower and rape seeds. The Xcc were not detected on 92(83.6%) of the *Brassica,* with 43.6% in cabbage, 21.8% of collards and 15.5% of kale. The least 1.8% of Xcc was detected in Chinese cabbage, 0.9% in cauliflower and none in rape seed. However 18 (16.4%) of the *Brassica* had Xcc detected. The findings indicated that the Xcc detected present varied among the types of *Brassica,* with kales having the highest number, followed by collards and cabbages.

Table 3 Type of *Brassica* seeds and incidence of Xcc

Type		**Xcc detected**		**Total**
		Not present	**Present**	
Cabbage	Count	48	2	50
	% of Total	43.6%	1.8%	45.5%
Cauliflower	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Chinese cabb.	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Collards	Count	24	2	26
	% of Total	21.8%	1.8%	23.6%
Kale	Count	17	13	30
	% of Total	15.5%	11.8%	27.3%
Rape seed	Count	0	1	1
	% of Total	0.0%	0.9%	0.9%
Total	**Count**	**92**	**18**	**110**
	% of Total	**83.6%**	**16.4%**	**100.0%**

In order to establish the relationship between type of *Brassica spp* and Xcc detected, cross tabulation Chi-Square was used. A significant chi-square test indicated that the data varied from the expected values, whereas a test that is not significant indicates that the data was inconsistent with the expected values. The cross tabulation chi-square was used to establish the relationship between type of *Brassica spp* and Xcc detected as summarized in Table 4.

Table 4 Chi-Square Tests on Type of *Brassica* seeds

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	28.655[a]	5	.000
Likelihood Ratio	26.093	5	.000
Linear-by-Linear Association	17.124	1	.000
N of Valid Cases	110		

a. 8 cells (66.7%) have expected count less than 5. The minimum expected count is .16.

From the findings it showed that there was a significant association between type of *Brassica spp* and Xcc detected (χ^2 = (28.66), (5) $p<0.05$. This was an indication that type of *Brassica spp* influenced and Xcc detected.

There is clear evidence there are differences between the types of *Brassica sp* regarding the Xcc detected and Chi-square was significant (p=0.000). Hence we reject the null hypothesis that type of *Brassica sp* does not influence Xcc detected.

4.1.2 Type of *Brassica* seeds and incidence of Xcc

From the study 18 cultivars of *Brassica* seeds were established as summarized in Table 5.

Table 5 Type of Cultivar and incidence of Xcc

Cultivar		Xcc detected		Total
		Not present	Present	
Copenhagen Market	Count	22	2	24
	% of Total	20.0%	1.8%	21.8%
Drum Head	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Gloria	Count	6	0	6
	% of Total	5.5%	0.0%	5.5%
Prize Drumhead	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Romenco	Count	3	0	3
	% of Total	2.7%	0.0%	2.7%
Sugarloaf	Count	4	0	4
	% of Total	3.6%	0.0%	3.6%
Victoria (Gloria)	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Georgia (Sukuma wiki)	Count	12	0	12
	% of Total	10.9%	0.0%	10.9%
Sukuma Wiki	Count	14	2	16
	% of Total	12.7%	1.8%	14.5%
Thousand Headed	Count	10	8	18
	% of Total	9.1%	7.3%	16.4%
F1	Count	4	0	4
	% of Total	3.6%	0.0%	3.6%
Supermaster	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kibo Giant	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Michihili	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Kinale	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Komolo	Count	3	6	9
	% of Total	2.7%	5.5%	8.2%
Golden Acre	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kopkool	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Total	**Count**	**92**	**18**	**110**
	% of Total	**83.6%**	**16.4%**	**100.0%**

Out of 110 incidences investigated, Copenhagen market represented the highest incidence of 24 (21.8%), it was followed by 18 (16.4%) thousand headed, 16 (14.5%) sukuma wiki, 12 (10.9%) of Georgia (sukuma wiki), 9 (8.2%) of Komolo and 6 (5.5%) of Gloria. The other cultivars had incidences of less than 5%. The Xcc were not detected in 92 (83.6%)

and not detected in 18 (16.4%) of the *Brassica.* Copenhagen market represented the highest incidence of 22 (20 %) Xcc not detected, it was followed by 14 (12.7%) of Sukuma wiki, 14 (10.9%) of Georgia (sukuma wiki). The other cultivars had no Xcc detected of less than 5.5%. The findings indicated that Xcc was not detected among the cultivars of Brassica, but detected on 8 (7.3%) thousand headed and 6 (5.5%) of Komolo.

The cross tabulation chi-square was used to establish the relationship between the cultivar of *Brassica sp and* the Xcc detected as summarized in Table 6. There was a relationship between the cultivar of *Brassica sp* and Xcc detected (χ^2 = (36.73), (17) $p<0.05$. This was an indication that cultivar of *Brassica* influenced Xcc detected. There was evidence that differences exist between the cultivar of *Brassica sp* with respect to the Xcc detected ($p<0.05$). Hence we reject the null hypothesis that cultivar of *Brassica sp* does not influence Xcc detected.

Table 6 Chi-Square Tests of Cultivar

	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	36.729[a]	17	.004
Likelihood Ratio	36.031	17	.005
Linear-by-Linear Association	9.857	1	.002
N of Valid Cases	110		

a. 30 cells (83.3%) have expected count less than 5. The minimum expected count is .16.

4.1.3 Region recorded the Highest Incidence of Xcc

During the study the research sought to establish the region that recorded the highest incidence of Xcc as shown in Table 7 (Appendix V). This was achieved by establishing the relationship between the region and Xcc detected. The Xcc were not detected on 92(83.6%) of the regions and detected in 18 (16.4%) of them. From the findings 4 (3.6%) of the Xcc incidences in Kehancha, 8 (7.3%) in Nakuru, 5 (4.5%) in Migori and 3 (2.7%) in Kongawea were not detected. The findings indicated that the Xcc was detected in Keumbo, region of collection. The cross tabulation chi-square was used to establish the relationship between the collection centre of *Brassica sp and* Xcc detected as summarized in Table 8. There was no significant relationship between the region of *Brassica sp collection* and Xcc detected (χ^2 = (55.199), (55) p>0.05. This was an indication that region of *Brassica* collection had no influence on Xcc detected.

Table 8 Chi-Square Tests on region and Xcc detected

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	55.199[a]	55	.467
Likelihood Ratio	53.983	55	.514
N of Valid Cases	110		

a. 109 cells (97.3%) have expected count less than 5. The minimum expected count is .16.

From the findings the chi square was not significant (P > 0.05), so we fail to reject the H_0. This means that we are not rejecting the hypothesis that the region and Xcc detected are independent (in other words, there is not a relationship between the region and Xcc

detected. This was attributed to the fact that samples used in the study were random selected throughout the country with no specific reference to ecological zones.

4.1.4 Seed Source and Xcc detected

From the study out of 110 incidences investigated, the Xcc were detected on 92 (83.6%) of the *Brassica,* and not detected on 18 (16.4%) as shown in Table 9.

Table 9 Seed Source and Xcc Detected

		Xcc detected		Total
		Not present	**Present**	
Amiran Kenya Ltd	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Bonanaza Seeds	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
E A Seeds	Count	30	1	31
	% of Total	27.3%	0.9%	28.2%
Farmer	Count	1	3	4
	% of Total	0.9%	2.7%	3.6%
Griffaton	Count	6	0	6
	% of Total	5.5%	0.0%	5.5%
Jumbo Agrovet (ex E A Seeds)	Count	2	2	4
	% of Total	1.8%	1.8%	3.6%
Kenya seed	Count	7	2	9
	% of Total	6.4%	1.8%	8.2%
Market	Count	4	5	9
	% of Total	3.6%	4.5%	8.2%
Royal Sluis	Count	7	1	8
	% of Total	6.4%	0.9%	7.3%
Service Plus	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Simlaw	Count	29	4	33
	% of Total	26.4%	3.6%	30.0%
Vet & Agron. Ltd (ex Griffaton)	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Total	**Count**	**92**	**18**	**110**
	% of Total	**83.6%**	**16.4%**	**100.0%**

Market represented the highest incidence of 5(4.5%), it was followed by 4(3.6%), Simllaw seeds, 3(2.7%) of the farmers seeds and 1.8% each of the Jumbo Agrovet and

Kenya seed. The findings indicated that Xcc was detected among some various seed source of *Brassica*.

The cross tabulation chi-square was used to establish the influence of *Brassica sp* seed source *and* the Xcc detected as summarized in Table 10. There was a significant relationship between the *Brassica sp* seed source and Xcc detected (χ^2 = (30.46), (11) $p<0.05$. This was an indication that *Brassica* seed source influenced Xcc detected. There was evidence that differences exist between the *Brassica sp* seed source with respect to the Xcc detected ($p<0.05$). Hence we reject the null hypothesis that *Brassica sp* seed source does not influence Xcc detected.

Table 10 Chi-Square Tests on Seed source

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	30.461[a]	11	.001
Likelihood Ratio	26.860	11	.005
N of Valid Cases	110		

a. 16 cells (66.7%) have expected count less than 5. The minimum expected count is .16.

4.1.5 Origin of *Brassica sp* and Xcc detected

The researcher sought to establish the influence of origin of *Brassica sp* on Xcc detected as summarized in Table 11. The Xcc were not present in 92(83.6%) and detected in 18 (16.4%) of them. From the findings 7(6.4%) of the Xcc incidences had its origin unknown, with 6 (5.5%) from Kenya, 2 (1.8%) from Netherlands and Denmark were not detected. The findings indicated that the Xcc was detected in Kenya, Netherlands and Denmark.

Table 11 Origin of *Brassica sp* and Xcc detected

		Xcc detected		Total
		Not present	**Present**	
Arusha, TZ	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Denmark	Count	15	2	17
	% of Total	13.6%	1.8%	15.5%
France	Count	12	0	12
	% of Total	10.9%	0.0%	10.9%
Kenya	Count	12	6	18
	% of Total	10.9%	5.5%	16.4%
Kinale	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Netherlands	Count	10	2	12
	% of Total	9.1%	1.8%	10.9%
Unknown	Count	26	7	33
	% of Total	23.6%	6.4%	30.0%
USA	Count	15	1	16
	% of Total	13.6%	0.9%	14.5%
Total	**Count**	**92**	**18**	**110**
	% of Total	**83.6%**	**16.4%**	**100.0%**

The cross tabulation chi-square was used to establish the influence of origin of *Brassica sp and* the Xcc detected as summarized in Table 12. There was a significant relationship between the origin of *Brassica sp* and Xcc detected (χ^2 = (8.55), (7) p<0.05.This was an indication that origin of *Brassica sp* influenced Xcc detected. From the findings the chi square was not significant (P > 0.05), so we fail to reject the H_0. This means that we are not rejecting the hypothesis that the region and Xcc detected are independent (in other words, there is not a relationship between the region and Xcc detected. This was attributed to the fact that samples used in the study were random selected throughout the country with no specific reference to ecological zones.

Table 12 Chi-Square Tests on Origin of *Brassica sp*

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.553[a]	7	.286
Likelihood Ratio	10.413	7	.166
N of Valid Cases	110		

a. 9 cells (56.3%) have expected count less than 5. The minimum expected count is .16.

4.2 Determination of the presence and survival of Xcc in Brassica crop debris

The second objective was to determine the existence and survival of *Xcc* in *Brassica* debris with respect to the type and soil debris. This was determined using descriptive statistics and analysis of variance. In occasions where the data has more than one dependent continuous variable or more than two values across the categorical independent variables, an Analysis of Variance (ANOVA) was used to compare the presence and survival of Xcc in Brassica crop debris.

4.2.1 Descriptive statistics on type of *Brassica* debris and Number of Xcc present

From the study, the mean number of colonies with respect to Brassica debris was varied as summarized in Table 13. The Kales had the highest mean number of colonies with Xcc of (33), compared to the cabbage with (29.94) and combined cabbage and kale (23.73). This indicated that the mean of Xcc present varied among the type of *Brassica* debris. The number of Xcc present showed that kale had the minimum (3) as well as the maximum (84) number of Xcc, with cabbages having a minimum of five and maximum of 68 Xcc.

Table 13 Descriptive statistics on type of *Brassica* debris and Number of Xcc present

Type of Brassica	N	Mean	Std. Deviation	95% Confidence Interval for Mean	Minimum	Maximum

				Lower Bound	Upper Bound		
Cabbage	17	29.9412	17.11896	21.1394	38.743	5.00	68.00
Kale	16	33.0000	20.17589	22.2490	43.751	3.00	84.00
Cabbage and Kales	11	23.7273	10.61217	16.5979	30.857	8.00	46.00
Total	**44**	**29.50**	**17.04099**	**24.3191**	**34.681**	**3.00**	**84.00**

A one-way analysis of variance was conducted to explore the *Brassica* debris and Number of Xcc present as shown in (Table 14). There was no statistically significant difference p>.05 in type of *Brassica* debris [*F* (2, 41) =973, p=.386] and number of Xcc present. Since the effects of u type of *Brassica* debris were found not to be significant, it implies that the means do not differ much.

Table 14 ANOVA on type of *Brassica* debris and Number of Xcc present

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	565.877	2	282.939	.973	.386
Within Groups	11921.123	41	290.759		
Total	**12487.000**	**43**			

4.2.2 Descriptive statistics on length of survival of Bacteria and Number of Xcc present

From the study, the mean number of colonies with respect to length of survival and number of Xcc present was varied as summarized in Table 15. The one month had the highest mean number of colonies with Xcc of (31), compared to the two months with (30) and three months (26). This indicated that the mean of Xcc present varied with length of

survival of bacteria. The number of Xcc present showed that the survival of Bacteria depend on the length of debris.

Table 15 Descriptive statistics on length of survival of Bacteria and Number of Xcc present

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
30.00	31.2000	13.82446	24.7300	37.6700	3.00	59.00
60.00	30.0000	17.89870	18.6277	41.3723	8.00	68.00
90.00	26.1667	21.61158	12.4353	39.8980	5.00	84.00
Total	29.5000	17.04099	24.3191	34.6809	3.00	84.00

The one-way Analysis of Variance was conducted to explore the effect of ANOVA on length of survival of bacteria and number of Xcc present as shown in (Table 16) There was a statistically significant difference $p<.05$ in length of survival of bacteria [F (2, 41) =27.08, p=.000]. Since the effects in length of survival of bacteria were found to be significant, it implies that the means differ more than would be expected by chance alone.

Table 16 ANOVA on length of survival of Bacteria and Number of Xcc Present

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	194.133	2	97.067	27.075	.000
Within Groups	12292.867	41	299.826		
Total	**12487.000**	**43**			

Brassica crop debris was visible and was collected from the plots up to 89 days from the start of the trial. After three months, debris was no longer visible and therefore only soil samples had pathogens. However after a month no *Xcc* were detected in the soil debris in all plots. This indicated that the survival of Xcc depends on the duration of existence of *Brassica* crop debris in the soil, but does doesn't depend on type of *Brassica.*

4.3 Races of *Xanthomonas Campestris* pv *campestris* found in Kenya.

The third objective was to determine the races of *Xanthomonas Campestris* pv *campestris* found in Kenya. This objective was achieved through establishing colony description of Xcc on artificial media and pathogenicity tests of isolates.

4.3.1 Colony description of Xcc on artificial media

The sample plates were examined for the presence of typical Xcc colonies on artificial media, after 3-4 days on FS selective media, Xcc colonies were small, pale green, mucoid and surrounded by a zone of starch hydrolysis. This zone appeared as a halo/circle of light (Figure 3 and 4) were visible on selective media FS after 3 days.

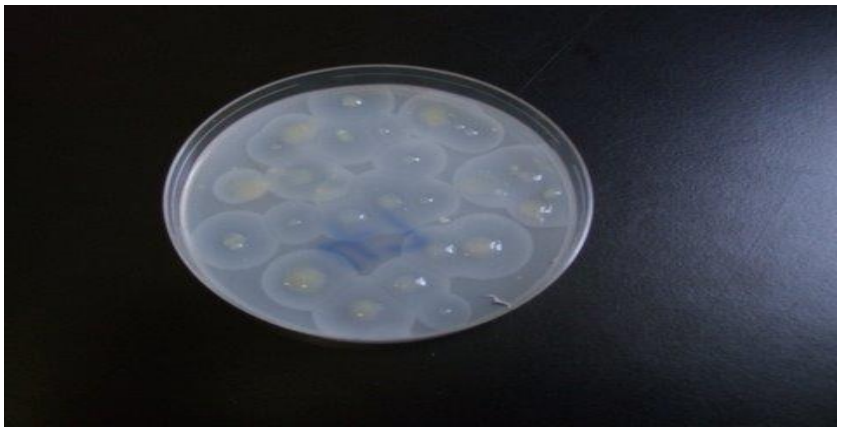


Figure 3: Pale yellow Mucoid (Xcc) and surrounded by a zone of starch hydrolysis

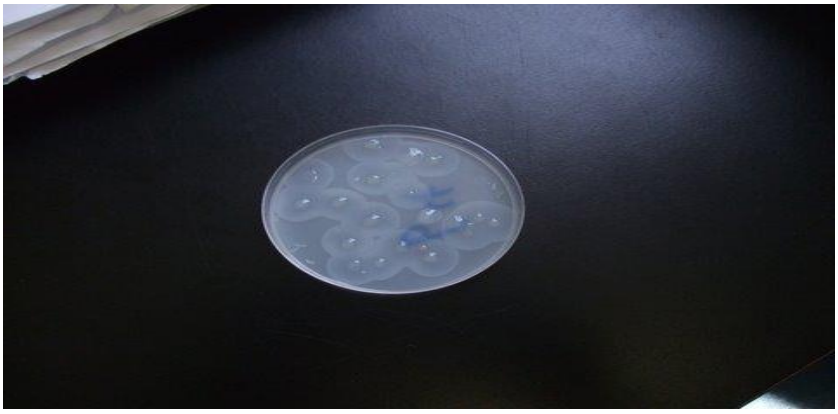


Figure 4: Pale green, Mucoid (Xcc) and surrounded by a zone of starch hydrolysis.

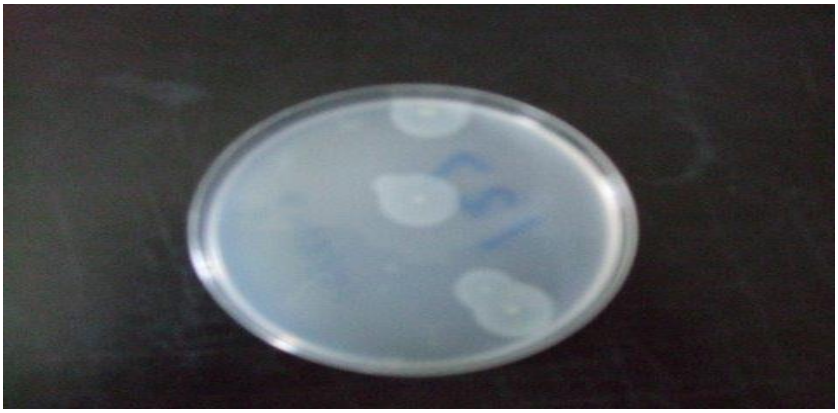


Figure 5: The Mucoid (Xcc) surrounded by the hydrolysis of starch but coalesce is minimum .

Also a zone appears as a halo for mCS 20 ABN selective media. The Xcc colonies were pale yellow, mucoid and surrounded by a zone of starch hydrolysis Figure 5.

Table 17 Tests for *Xanthomonas Campestris pv. campestris* on samples of *Brassica* seed available to farmers in Kenya

Type	Tested	Positive	Countries of origin
Cabbage	50	3	Denmark, France, Unknown
Cauliflower	1	0	Unknown
Chinese cabbage	2	0	Unknown
Collards	26	7	Kenya, USA, Unknown
Kale	30	12	Denmark, France, Unknown
Rape	1	1	Unknown
Totals	**110**	**23**	

4.3.2 Pathogenicity Tests of isolates

The inoculum isolated from the *Brassica* seeds purchased throughout Kenya from seed merchants, stockists, hawkers and open air markets were kept in the fridge at -80 degrees Celsius as it awaited inoculations into the susceptible Savoy cabbage wirosa FI, the seedlings from wirosa seeds imported from United Kingdom were planted in trays in the greenhouse as shown in Figure 6 giving symptoms of typical disease.

Plate A: These were the susceptible Savoy cabbage wirosa planted for pathogenicity

Plate B: Wirosa seedling inoculated with Xcc by pricking veins. Infection along the midrib

Plate C: Inoculated with Xcc by pricking veins. There was infestation of black rot which meant that the isolates were pathogenic

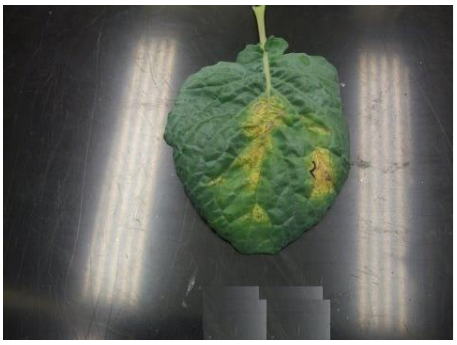


Plate D: Blackening veins, typical symptom of black rot

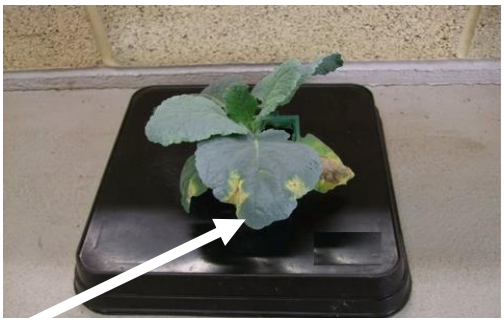


Plate E: Wirosa seedling inoculated with Xcc by pricking veins. Typical V-shape

Source: Author 2015

Figure 6: Pathogenic Symptoms of Typical Disease

Three weeks after sowing, the Isolates were grown on YDC (Yeast extract-dextrose-CaCO3) medium which is a non-selective media at 28- 30°C for 48 hours before inoculation. Based on the relative size of the largest lesion on the leaf: 0, no symptoms; 1, slight necrosis or chlorosis surrounding the infection point; 2, typical V-shaped yellow or necrotic lesion with blackened veins with a lesion size of less than 1 cm square, 2cm square, and 3cm, square typical V-shaped lesion with a size of more than 1 cm^2. Several re-isolations were performed to determine the presence or absence of bacteria which resulted into race typing of the isolates. Out of one hundred and ten seed samples tested, twenty three isolates were found to be pathogenic on Savoy cabbage cv Wirosa F1 giving symptoms typical of the disease, V-shaped, yellow lesions with black veins and usually with necrotic centers.

These isolates were considered to be *X. campestris* pv. *campestris*. The 110 seed samples tested, including 50 cabbage, 1 cauliflower, 2 Chinese cabbage, 26 collards, 30 kales and 1 rape. *Xcc* was detected in twenty three samples representing each of the three major *Brassica* types (cabbage, collards, kale). In both home-produced seed lots and seed lots imported from Denmark, France and the USA; and in seed obtained both from reputable suppliers and more dubious sources.

Table 18 List of Differential cultivars

1. Wirosa F1	***Brassica oleracea***
2. Just Right Hybrid Turnip	*Brassica rapa*
3. Cob60	*Brassica napus*
4. Seven Top Turnip	*Brassica rapa*
5. Bo99075 (PI 199947)	*Brassica corinata*
6. FBLM2	*Brassica juncea*
7. Miracle F1	*Brassica oleracea*
8. SxD1	*Brassica oleracea*

In a number of cases multiple samples of seed labelled with the same seed lot number were obtained from different places and suppliers; assuming that in most cases the lot numbers were correct estimates of infection levels were derived from the combined results of all samples of the same seed lot. The same seed lot numbers of the samples collected was mixed and only one sub sample analyzed. Some seeds were labelled with the same seed lot numbers which means they were not genuine seeds. Determination of races of Xcc in Kenya was done using the reaction of differential cultivars (List down the differential cultivars as highlighted in the methodology. Six races of *Xanthomonas Campestris* pv. *campestris* have been identified on the basis of their reactions on a series of differential *Brassica* genotypes. Races 1 and 4 are the most important races worldwide (Table 19).

The reaction of the differential cultivars described by Kamoun *et al*; (1990) was used for the initial separation of *X. campestris* pv. *campestris* isolates into races. Isolates corresponding to Kamoun's races 0, 1, 2, 3 and 4 were identified among those tested, but isolates showing the pattern of reaction of Kamoun's race 0, 2 and 4 were not found. The reaction of the differential cultivars described (Kamoun *et al* 1990) was used for the separation of *X. campestris* pv. *campestris* isolates into races. Isolates corresponding to Kamoun's races isolates showing the pattern of reaction of Kamoun's race 0, 2 and 4 were not found, only race 1 and 3 were observed.

Table 19: Xcc races found in Kenya using the reaction of the differential cultivars described by Kamoun *et al*; (1990)

Type	Cultivar	Collection centre	Origin	Xcc detected	Savoy cabbage	Race type
Cabbage	Copenhagen Mkt	Nyahururu	-	Yes	+ve	3
Cabbage	Copenhagen Mkt		France	Yes	+ve	3
Cabbage	Supermaster	Subukia	Denmark	Yes	+ve	1
Collards	Georgia (Sukuma Wiki)		Kenya	Yes	+ve	1
Collards	Georgia (Sukuma Wiki)	Gucha	USA	Yes	+ve	1
Collards	Georgia (Sukuma Wiki)	Kehancha	USA	Yes	+ve	1
Collards	Georgia (Sukuma Wiki)	Sondu	USA	Yes	+ve	1
Collards	Sukuma Wiki	Naro-moru	-	Yes	+ve	1
Collards	Sukuma Wiki	Timborua/Iten	-	Yes	+ve	1
Kale	Th. Headed	Isebania	-	Yes	+ve	1
Kale	Th. Headed	Nakuru	France	Yes	+ve	1
Kale	Th. Headed	Karatina	France	Yes	+ve	3
Kale	Th. Headed	Kericho	Denmark	Yes	+ve	3
Kale	Th. Headed	Muserechi	-	Yes	+ve	
Kale	Th. Headed	Mombassa	-	Yes	+ve	3
Kale	Th. Headed		Denmark	Yes	+ve	1
Kale	Th. Headed	Olkalau	-	Yes	+ve	1
Kale	Unknown	Kipisorwet	Kenya	Yes	+ve	1
Rape seed	Unknown	Njoro	-	Yes	+ve	1
Kale	Unknown	Nakuru	Kenya	Yes	+ve	1
Kale	Unknown	-	Kenya	Yes	+ve	1
Kale?	Unknown	Keumbo	Kenya	Yes	+ve	1
Kale	Unknown	Keumbo	Kenya	Yes	+ve	1

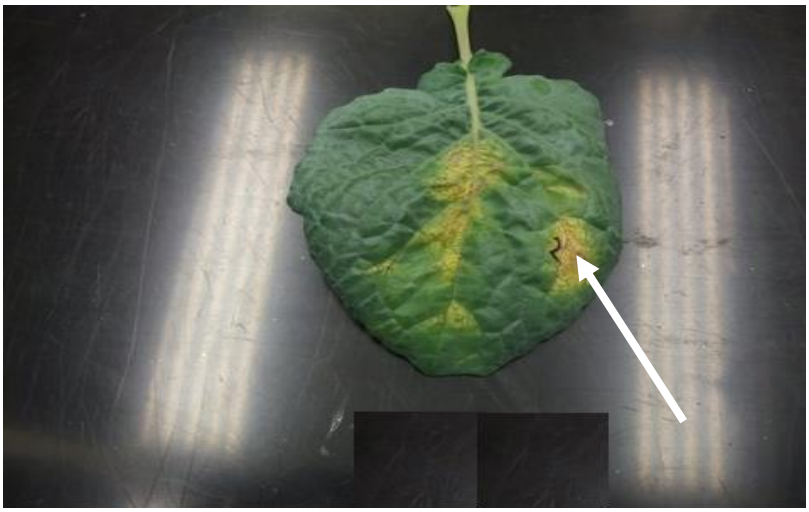
Race type 1 and 3 were isolates showing the reaction of the differential cultivars described by Kamoun *et al*; (1990).

The number of infected points per leaf and the severity of symptoms were assessed they were rated on a scale of 0 to 4 (Kamoun's *et al.,* 1990). This was based on the relative size of the largest lesion on the leaf: 0, no symptoms; 1, slight necrosis or chlorosis surrounding the infection point; 2, typical V-shaped yellow or necrotic lesion with

blackened veins with a lesion size of less than 1 cm 2, and 3, typical V-shaped lesion with a size of more than 1 cm^2 as shown in Figure 7 and 8.

Source: Author 2015

Figure 7: Chlorosis surrounding the infection point



Source: Author 2015

Figure 8: Typical V-shaped lesion along the midrib of the seedling

CHAPTER FIVE

DISCUSSION

5.1 Incidence of Xcc in Brassica seeds obtained from various sources in Kenya

The first objective was to assess the occurrence of *Xanthomonas Campestris* pv *campestris* in *Brassica* seeds available for production in Kenya. The Xcc detected varied among the types of *Brassica*, with kales having the highest number, followed by collards and cabbages. From cross tabulation Chi-Square there was a significant association between type of *Brassica sp* and Xcc detected (χ^2 = (28.66), (5) $p<0.05$. This was an indication that type of *Brassica sp* influenced and Xcc detected. This agrees with Toit *et al.,* (2005) on incidence of *Xanthomonas Campestris* pv *carotae* on carrot seed irrigated by drip or sprinkler in central Oregon, (infested with 106 to 108 colony forming units (CFUs) of Xcc per 10,000 seeds generally compares well with the finding in the incidence of Xcc in *Brassica* seeds in Kenya.

The Xcc was not detected among the cultivars of *Brassica,* but detected on 8 (7.3%) thousand headed and 6 (5.5%) of Komolo. There was a relationship between the cultivar of *Brassica sp* and Xcc detected (χ^2 = (36.73), (17) $p<0.05$. This was an indication that cultivar of *Brassica* influenced Xcc detected. The Xcc was detected in Keumbo, region of collection. There was no significant relationship between the region of *Brassica sp* collection region and Xcc detected (χ^2 = (55.199), (55) $p>0.05$. This was an indication that region of *Brassica* collection had no influence on Xcc detected. The Xcc was detected among some various seed source of *Brassica*. Differences exist between the *Brassica sp* seed source with respect to the Xcc detected. The findings indicated that the

Xcc was detected in Kenya, Netherlands and Denmark. According to Raymond *et al.*, (1987) on association of Xcc with plants of *Brassica* agrees with the findings both on *Brassica* crop debris and the seeds. According to Poplawsky and Chun (1989) their concern was on the symptoms of the two pathovars of Xcc. Groot *et al.,* (2005/2008) interest was on the physical sanitation and seed maturity as a control against disease infestation.

Based on the results, the presence of *Xcc* in vegetable *Brassica* seed available to farmers in Kenya is widespread and should be a cause for considerable concern. The frequency of occurrence is very much higher than in vegetable *brassica* seed sold in developed countries and much higher than would be expected if seed companies/suppliers were taking any precautions to avoid supplying infected seed. It should be noted that for many of the samples tested, only a single sub-sample of seeds was tested, not several sub-samples and implies that the results were from only one sample in all the tested seeds. The frequent occurrence of *Xcc* in samples of collards and kales imported from developed countries is a clear indication that these seeds are not being tested for *Xcc* in the countries of origin.

In many cases, the labelling of seed containers was not adequate to identify the country of origin of the seed or the precise chemical treatment regardless of any legal or statutory obligations, the absence of such information should be considered to be bad practice and regulation should capture this so that all information is on the labelled packaging materials. In addition, the lot numbers appearing on the containers of many samples did

not appear to comply with official requirements, bringing into question other information on the labels and in the cases of imports, whether they had been imported legally. Nearly all of the seeds examined appeared to have been treated with a fungicide, although the chemical used was often not indicated on the container. Such fungicidal seed treatments will have little or no effect on *Xcc*, a bacterial pathogen, and as such may be giving importers, officials and growers a false sense of security with respect to the health status of *Brassica* seed.

5.2 Determination of the presence and survival of Xcc in Brassica crop debris

The second objective was to determine the existence and survival of *Xcc* in *Brassica* debris with respect to the type and soil debris. The number of Xcc present showed that kale had the minimum (3) as well as the maximum (84) number of Xcc, with cabbages having a minimum of five and maximum of 68 Xcc. There was no statistically significant difference $p>.05$ in type of *Brassica* debris [F (2, 41) =973, p=.386] and number of Xcc present. The effects of u type of *Brassica* debris were found not to be significant, it implies that the means do not differ much.

The one month had the highest mean number of colonies with Xcc of (31), compared to the two months with (30) and three months (26). This indicated that the mean of Xcc present varied with length of survival of bacteria. The number of Xcc present showed that the survival of Bacteria depend on the length of debris. There was a statistically significant difference $p<.05$ in length of survival of bacteria [F (2, 41) =27.08, p=.000]. The length of survival of bacteria was found to be significant an. *Brassica c*rop debris was visible during the start of the trial and after three months, debris was no longer

visible. The numbers of *Xcc* was not detected then declined as identifiable debris became no longer visible in the plots, after 90 days. This implies that the Xcc reduced with the length of the survival of debris.

The survival of Xcc depends on the duration of existence of *Brassica* crop debris in the soil, but does doesn't depend on type of *Brassica. This agrees with* Kocks *et al.,* (1998) Survival and Extinction of *Xanthomonas Campestris pv. campestris* in Soil infested with infected plant debris proceeded exponentially and extinction rates depended on temperature, as did the decomposition of plant debris. In replicated field plots, over three years, this work was done in Netherlands. I would attribute the six months duration of pathogen in the soil to the high rate of decomposition due to high temperatures in Kenya (Zhao *et al.,* 1999/2000).

The bacterium that causes black rot overwinters on and in seed and crop debris left in the field. One to three infectd seeds among 10,000 seeds could result in an outbreak of black rot, if conditions are conducive for black rot development. The organism survives especially well in cabbage and brussels sprout refuse and in many weeds. Bacteria are spread by splashing or flowing water, blowing of detached leaves or dust particles, shipping and handling of infected plants, and insect feeding. The optimum conditions for growth of the organism are temperatures from 77 to 86 F and free moisture in the form of dew, fog, or rain.

These results clearly indicate that large numbers of *Xcc* may survive for several months in *Brassica* crop debris, regardless of whether it is incorporated into the soil or left on the surface. There was no difference in all the trials because pathogen disappeared at the same time, however, once the debris degraded, the numbers of *Xcc* declined and became undetectable in the soil. The very large numbers of *Xcc* which were present in the debris suggest that debris from infected crops presents a very high risk to subsequent crops planted in or near it, so long as the debris remains. *Xcc* appears to survive poorly in the soil itself and therefore once visible debris has been absent for several months there would appear to be little risk. Thus any actions which can be taken to increase the rate of breakdown of *Brassica* debris would appear to be beneficial.

In terms of disease management, these results suggest, that if infected crop debris is left in the field, whether incorporated or not, that *brassica* should not be planted into the same or nearby fields for at least 6 months. It is recognised that such an interval between crops would be inevitable for farmers; therefore it is an alternative approach to dealing with infected or potentially infected debris. At first sight, chopping the debris as small as possible and incorporating into the soil may encourage more rapid breakdown, but conversely this may increase the short term risks resulting from highly contaminated equipment and people, and the potential for raising infectious dust if the debris is dry, or aerosols if wet.

The most effective way of dealing with infected crop debris could be to remove from the field and then effective aerobic composting which achieves high temperatures and rapid

breakdown of the material. Burning of dry debris may be another option. In order to monitor the survival of *X. campestris* pv. *armoraciae* and *P. syringae* pv. *maculicola* in the field, turnip and collards were inoculated with rifampicin-resistant strains and were buried beneath the soil or left on the soil surface. Both pathogens were recovered from turnip and collard debris up to 2 months following burial, but neither pathogen was recovered from soil after the debris had decomposed. However, both pathogens were recovered from plant debris left on the soil surface for up to 5 months.

5.3 Determination of races of Xcc in Kenya

Pathogenicity tests performed by inoculating leaves of known susceptible Wirosa FI/Differential cultivars (table seedlings, infection symptoms induced were categorized into four groups. Xcc isolates from the seed stock tested compared well with Kamoun's *et al* (1990). The number of infected points per leaf and the severity of symptoms were assessed and rated on a scale of 0 to 4 based on the relative size of the largest lesion on the leaf race type 1 and 3 were obtained. There was no race 0, 2 and 4 Vicente *et al.* (2001) reaction methods confirms these results but the method of Xcc scoring/ scale is 1-9 and the method of inoculation is by use of mouse tooth forceps. Which do a lot of points around the margin of the leaves in the secondary veins. When a plant is attacked by the pathogen, it can defend itself against the infection by recognizing the virulence genes of the pathogens and mount defence responses from the complimentary resistance genes (Staskawicz *et al.*, 1995); Lamb *et al.*, 1989).

Recognition of the pathogen by resistant plant triggers the activation of plant defense that results in the halting of pathogen ingress. The term virulence is commonly used in plant

pathology to genetically define the inability of the pathogen to cause disease on a resistant host plant. When a plant does not have a corresponding resistance gene a compatible reaction (Suscetpability) occurs. Race identification in *Xanthomonas Campestris pv campestris* was carried out using differential cultivars. This agrees Williams, (1980) in implementation of an integrated disease management using host specific resistance in production regions.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The Xcc detected varied with the type of Brassica, cultivar and seed source. The kales had the highest number of Xcc, followed by collards and cabbages. The type of *Brassica sp*, cultivar, and *seed* source influenced the Xcc detected. The Xcc detected was not influenced by collection region and origin of *Brassica.*

The number of Xcc present in kale was slightly more than those found in cabbages. However there was no statistically significant difference on the number of Xcc present. The number of Xcc present in the debris depends on the existence of debris and the survival of bacteria decreases with increase in duration of exposure. The survival and existence of Xcc present in the soil was significant with time. The Xcc in the soil reduced with the length of time it is exposed.

The Xcc isolates from the seed stock tested and rated on a scale of 0 to 4 based on the relative size of the largest lesion on the leaf race type 1 and 3 were obtained. There was no race 0, 2 and 4. Recognition of the pathogen by resistant plant triggers the activation of plant defense that results in the halting of pathogen ingress.

6.2 Recommendation

The seeds available for farmers in Kenya are infested with *Xanthomonas Campestris pv campestri*s and has no cure since the seeds dressed with fungicides cannot manage the

bacteria, as the results depicted clearly. Therefore it would be important to advise on the seed regulations in this country and seeds available should be certified to avoid infestation and spread of the black rot disease.

The *Brassica* debris left on the farms are a source of inoculum, therefore it is advisable to remove and/or burn all the *Brassica* debris to avoid the carry-over of the inoculum. The possible future researchable areas to check on the incidence of the inoculum are the effect of different Agro ecological zones, Rainfall and temperature regimes. Also planting of healthy *Brassica* plants on experimental plots and Bait plants could be planted after the 2 negative results to show that after an appropriate interval, it is safe to plant *Brassica* vegetables on the same area.

There is a need to produce seed in disease free regions where there is no infested plant debris. Tests seeds for presence of pathogen and only use clean seed lots. The use of clean seed is significant and extremely effective means of disease control for seed borne diseases. Management should start from national, regional and at the farm level for effective reduction and finally eradication. It is very important to purchase only certified, pathogen-free seed and disease-free transplants. Grow plants in fields where a crucifer crop has not been grown for at least three consecutive years. Some cabbage, rutabaga, turnip, kale, and black mustard varieties are available that have varying degrees of resistance to black rot. Apply recommended bactericides when conditions favor disease.

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APPENDIXES

Appendix 1: Methods and Materials

Extraction Methods

Extraction methods for *Xanthomonas Campestris pv campestris* (Black rot).

The Brassica seeds for extraction of Xcc are subjected to series of seed tests of which the maximum sub sample size should be 10,000 seeds that formed the working sample.

Materials:

1. Reference material known strain of Xcc.
2. Plates of FS medium 9.0cm Petri dishes.
3. Plates of mCS20 ABN medium 9.0 cm petridishes
4. Plates of YDC for sub-culture
5. Conical flasks of sterile saline (0.85%) Nacl + tween 20 (0.02%)
6. Dilution bottles containing 4.5ml of sterile saline
7. 70% Ethanol for disinfection of surfaces, equipments.
8. Incubator operating at 28-30^{0}c
9. Auto matic pipettes
10. Brassica seedlings susceptible to all races of the pathogens e.g. wirosa for pathogencity test.
11. Brassica seeds for seed tests and extraction Xcc.
12. Orbital shaker
13. Sterile pipette tips
14. Sterile bent glass rods
15. Micro centrifuge

16. Sterile 1.5 ml micro centrifuge tubes.

Preparation of media: FS, mCS20 ABN AND YDC.

Two different media used to extract Xcc i.e. FS and mCS20 ABN because they have a different spectrum of antibiotics i.e. gentamycin in FS can inhibit Xcc while some strains of Neomycin in mC20 ABN can inhibit Xcc as well. However two media is like assurance and methyl green inhibits gram-positive bacteria.

Preparation of mCS20 ABN: -

Compound	G/l	**G/500ml**
Soya peptone (oxoid L44)	2.0	1.0
Bacto tryptone (Difco)	2.0	1.0
KH_2PO_4	1.587	0.794
$(NH_4)_2HPO_4$	0.330	0.165
$MgSo_4\ 7H_2o$	0.4	0.2
L. Glutamine (Sigma G 3126)	6.0	3.0
L-Histidine (Sigma H-8000)	1.0	0.5
D-Glucose (Dextrose)	1.0	0.5
Distilled/de-ionised water	1000ml	500ml
Bacto agar (Difco) soluble	15.0	7.5
Soluble starch (Aldrich No. 17, 993-0) (CCP)	10.0	5.0
*Cycloheximides (200mg/ml 70% EtOH)	200mg (1ml)	100mg (0.5ml)
*Bacitracin (100mg/ml 50% EtOH)	100mg (1ml)	50mg (0.5ml)
*Neomycin (40mg/ml 20% EtOH)	40mg (1ml)	20mg (0.5ml)

NB: Antibiotics indicated by * are added after autoclaving

Weigh out all ingredients except starch, agar and antibiotics into a suitable container.

1. Add the distilled/de-ionised water.
2. Dissolve and check, PH which should be 6.55, adjust if necessary important critical control point.
3. Add starch and agar then steam to dissolve.
4. Autoclave at 121 degrees centigrade, 115 Psi for 15 minutes.
5. Prepare antibiotic solution if necessary.

6. Allow medium to cool to approximately 50 degrees centigrade and aseptically add antibiotics solution.
7. Mix thoroughly but gentle to avoid air bubbles using a magnetic stirrer bar or by inversion/swirling and pour plates (22 mls per 9.00 cm plate).
8. Leave plates to dry in a Laminar flow bench.

Antibiotic stock solutions amount s in mCS20 ABN.

Cycloheximides – dissolve 2.0g in 10ml 70% ethanol. Add0.5ml/500ml.

Neomycin – dissolve 400mg in 10 ml 20% ethanol add 0.5ml/500ml.

Bacitracin – dissolve 1.0g in 10ml 50% ethanol add 0.5 ml/500ml.

The prepared plates of medium are inverted and stored in polythene bags at 4 degrees centigrade and used within two weeks of preparation to ensure activity of antibiotics.

Preparation of FS Media

Compound	**g/l**	**g/500**
Bactro agar	15.00	7.5
Soluble starch (Aldrich No. 17,993-0) CCP	10.00	5.0
Bactro yeast extract (Difco)	0.1	0.05
K_2HP0_4	0.8	0.4
K_2HP0_4	0.8	0.4
Mg SO_4. $7H_2O$	0.1	0.05
Methyl / green (1% ag)	1.5ml	0.75ml
Distilled / de-ionised water	1000ml	500ml
*Cycloheximides (200mg/ml 70% EtOH)	200mg (1ml)	100mg (0.5ml)
*D-methiomne (3mg/ml 50% EtOH)	3mg (1ml)	1.5mg (0.5ml)
*Pyridoxine HCL (1mg/ml 50% EtOH	1mg (1ml)	0.5mg (0.5ml)
*Cephalexin (50mg/ml 70% EtOH	50mg (1ml)	25mg (0.5 ml)
* Gentamycin (1mg/ml H_2O)	0.4mg (0.4ml)	0.2mg (0.2ml)
*Trimethoprim (10mg/ml 70%EtOH)	30mg(3ml)	15mg (1.5 ml)

NB: Antibiotics indicated by * are added after autoclaving

Weigh out all ingredients except antibiotics and methiomine into a suitable container.

1. Add 1000ml (or 500ml) of distilled/de-ionised water.
2. Steam to dissolve.
3. Auto clave at 121 degrees centigrade, 115 Psi for 15 minutes.
4. Prepare antibiotics and methiomine solutions and filter sterilize as appropriate.
5. Allow medium to cool to approx. 50 degrees centigrade before adding antibiotics and methiomine solution.
6. Mix thoroughly but gently to avoid air bubbles using a magnetic stirrer bar or by inversion /swirling and pour plates (2ml per 9.0cm plate)
7. Leave plates to dry in a laminar flow bench.

Antibiotic stock solution amounts in FS medium.

- Dissolve 2g Cycloheximides in 10ml 70% ethanol add 0.5ml/500ml.
- Dissolve 60mg D-methiomine in 10ml-distilled water, and then add 10ml ethanol. Add 0.5ml/500ml.
- Dissolve 20mg pyridoxine in 20ml 50% ethanol add 0.5ml/500ml.
- Dissolve 500mg cephalexin in 10ml 70% ethanol add 0.5ml/500ml.
- Dissolve 10mg gentamycin in 10ml distilled water, filter sterilize. Add 0.2ml/500ml.
- Dissolve/suspend 200mg trimethoprim in 20ml 70% ethanol. If it doesn't dissolve fully, vortex the suspension immediately before adding to the medium. Add 1.5ml/500ml.

Store prepared plates inverted in polythene bags at 4 degrees centigrade and use within two weeks of preparation to ensure activity of antibiotics.

Preparation of yeast dextrose chalk (YDC) agar medium.

This medium is used for routine culture of number of bacteria but especially Xanthomonas spp, which produce, characteristic yellow mucoid/fluidal growth.

Compound	**G/l**	**G/500**
Bactro agar	15.0	7.5
Yeast extract	10.0	5.0
CaCo3 (light powder)	20.0	10.0
D. Glucose (dextrose)	20.0	10.0
Distilled/de-ionised water	1000ml	500ml

1. Weigh out all ingredients into a suitable container. Use oversize container i.e. 250 ml of medium in a 500ml bottle (flask) to facilitate swirling of medium just before pouring
2. Add 1000ml (or 500ml) of distilled/de-ionised water.
3. Steam to dissolve (CCP)
4. Autoclave at 121 degrees centigrade 115 Psi for 15 minutes (CCP).
5. Allow medium to cool to below 50 degrees centigrade (CCP).
6. Swirl to ensure even distribution of CaCo3 and avoid air bubbles and pour plates (22ml per 9.0 cm plate).
7. Leave plates to dry in a laminar flow bench

Prepared plates are stored inverted in polythene bags at room temperature for several months provided they do not dry out.

Critical control points all ingredients should be completely dissolved before autoclaving and should not be overcooked to avoid caramelisation of glucose, which is indicated by darkening of the medium.

Preparation of sterile saline 0.85% saline:

Sterile 0.85% saline is routinely used as diluents in preparation of dilution for range of bacteria.

Compound	**G/l**	**G/500**
Sodium chloride (Nacl)	8.50	4.25
Distilled/de-ionised water	1000ml	500ml

1. Weigh out ingredients into a suitable container.
2. Add 1000ml (or 500 ml) of distilled / de-ionised water.
3. Dissolve and dispense into final containers.
4. Autoclave at 121 degrees centigrade, 115Psi for 15 minutes.
5. For extraction of seeds, add 20 ul of sterile tween 20 per 100ml after autoclaving. Can be stored for several months provided containers are tightly closed.

Preparation of seeds for extraction of Xcc

Each seed lots of 30,000 seeds are allocated unique sequential sample No. i.e. 782 and details recorded i.e. source, lot no TSW, seed treatments and each sub sample of a lot is also given a No. i.e. 782/1. Meaning seed lot No. 782 sub sample 1. Then should only be one 782/1 regardless of the test dates. Each colony sub-cultured from a sub sample should also be given a number which should be unique within the sub-sample, regardless of dilution e.g. 782/1/1 means lot 782, sub-sample 1, colony 1, colonies 1-3 could come

from one dilution and colonies 4-6 could come from another dilution records where they have come from by writing the colony no's on the dilution plates.

Details of Xcc extraction and preparation of seeds: -

Extraction of Xcc is prepared as follows: -

Procedure: -

1. Suspend seeds in pre-chilled (2-4 degrees centigrade) sterile saline plus tween 20 (0.02%) in a conical flask. The volume of saline should be adjusted according to the No. of seeds used (10ml per 1,000 seeds).
2. Shake for 5 min at room temperature (20-25 degrees centigrade) on an orbital shaker set at 100-150 rpm.
3. Pipette two 1 ml samples of the seed extract into two separate sterile micro-centrifuge tubes and keep on ice or in the refrigerator until processed.
4. Continue to shake the flask at 100-150 rpm at room temperature for 2.5hours.

Centrifugation: -

1. Centrifuge the two 1 ml samples of extract at 11,600 for 10 minutes.
2. Remove 0.8 to 0.9 ml from each of the micro-tubes taking care not to disturb the pellets.
3. Vortex to re-suspend the pellets in the remaining liquid.
4. Keep on ice or in the refrigerator until plated.
5. Pipette the entire volume (100-200 ul) of each of the re-suspended pellets onto plates of each of the selective media (FS, mCS20 ABN) and spread over the surface with a sterile bent glass rod.
6. Incubate plates

7. Shake the flasks (from step 4 above) to mix just before dilution.
8. Prepare two serial ten-fold dilutions from the seed extract. Pipette 0.5ml of the extract into 4.5ml of sterile saline and vortex to mix (10 dilution). Pipette 0.5ml of the 10 dilutions into another 4.5ml of sterile saline and vortex to mix (10^2 dilution).
9. Pipette 100 ul of each dilution and the undiluted seed extract onto plates of each of the selective media (FS, mCS20 ABN) and spread over the surface with a sterile bent glass rod.
10. Incubate plates at 28-30^0c and examine after 3-4 days.

Positive control (Culture or reference material)

1. Prepare a suspension of a known strain of Xcc in sterile saline.
2. Dilute sufficiently to obtain dilutions containing approx. 10^2 to 10^4 cfu/ml.
3. Prepare 100 ul of appropriate dilutions onto plates of each of the selective media (FS, mCS 20 ABN) and spread over the surface with a sterile bent glass rod. Incubate plates with the sample plates.

Sterility check: -

1. Prepare a dilution series from a sample of the extraction medium i.e. saline + tween 20 containing no seeds and plate on each of the media as for samples.

Examination of the plates: -

1. Examine sterility check and positive control plates (CCP).
2. Examine the sample plates for the presence of typical Xcc colonies by comparison with positive control plates.

3. On Fs after 3-4 days, Xcc colonies are small, pale green, mucoid and surrounded by a zone of starch hydrolysis. This zone appears as a halo that may be easier to see with a black background. Colonies may show variation in size and may be visible on Fs after 3 days.
4. After 3-4 days on FS, mCS 20 ABN, Xcc colonies are pale yellow, mucoid and surrounded by a zone of starch hydrolysis, colonies may show marked variation as in size as well.
5. Record the No. Of suspects.

Confirmation / identification of suspect colonies:

1. Sub-culture suspect colonies to sectored plates of YDC medium if present at least six colonies should be subculture per sub-sample.
2. Sub-culture the positive control isolate to a sectioned plate for companion.
3. Incubate sectioned plates for 24 – 48 hours at 28-30 degree centigrade.
4. Compare appearance of growth with positive control.

Pathogenicity:

The identity of isolates is confirmed by pathogencity on Brassica seedlings of known susceptibility. Grow seedlings of a Brassica cultivar known to be susceptible to all race of Xcc (e.g. cabbage CV wirosa) in small pots until at least 2 – 4 leaf stage. Inoculate with small amount of bacterial growth with sterile insect pin on the two youngest leaves on the major veins. Inoculate with positive control isolate grow on plants at 20-25^{0}c. Examine plants for the appearance of typical progressive V-shape, yellow necrotic lesions with blackened veins after 7 – 14 days.

Appendix II: Incidence of *Xanthomonas Campestris pv campestris* in *brassica* seed stocks obtained from seed lot in Kenya

Table V

Tested	Samp.	Type	Cultivar	Collection centre	Supplier	Lot No.	Origin	Treat	No tested	Xcc detect
June' 2008	112	Cabbage	Copenhagen Mkt	Kongowea	E A Seeds	03-698/307092	-	?	7,105	No
June' 2008	92	Cabbage	Copenhagen Mkt	Matuu	E A Seeds	04-7427/407064	-	?	9,940	No
June' 2008	39	Cabbage	Copenhagen Mkt	Busia	Amiran	102	France	TMTD	10,000	No
June' 2008	86	Cabbage	Copenhagen Mkt	Nyahururu	Simlaw	102	France	TMTD	10,000	No
June' 2008	117	Cabbage	Copenhagen Mkt	Malindi	Amiran Kenya Ltd	102	France	TMTD	11655	No
June' 2008	94	Cabbage	Copenhagen Mkt	Machackos	Vet & Agron. Ltd (ex Griffaton)	172	France	TMTD	9,695	No
June' 2008	14	Cabbage	Copenhagen Mkt	Migori	Kenya seed	311	-	-	10,000	No
June' 2008	2	Cabbage	Copenhagen Mkt	Kericho	E A Seeds	32060	Denmark	Thiram	10,000	No
June' 2008	16	Cabbage	Copenhagen Mkt	Kehencha	Simlaw	181400	Kenya	-	10,000	No
June' 2008	48	Cabbage	Copenhagen Mkt	Mumias	E A Seeds	406064	-	?	10,000	No
June' 2008	3	Cabbage	Copenhagen Mkt	Awendo	Royal Sluis	641187	Netherlands	Thiram	10,000	No
June' 2008	35	Cabbage	Copenhagen Mkt	Baitany	Royal sluis	641187	Netherlands	Thiram	10,000	No
June' 2008	62	Cabbage	Copenhagen Mkt	Nakuru	Griffaton	-	France	TMTD	10,000	No
June' 2008	86	Cabbage	Copenhagen Mkt		E A Seeds	022922	USA	?	1 x 10,000, 2 x 4,400	No
June' 2008	46	Cabbage	Copenhagen Mkt	Malaba	E A Seeds	03-698/307092	-	?	10,000	No
June' 2008	33	Cabbage	Copenhagen Mkt	Sondu	E A Seeds	046165-1	-	?	10,000	No
June' 2008	65	Cabbage	Copenhagen Mkt	Moi's Bridge	E A Seeds	04-7427/407064	-	?	2 x4,400	No
June' 2 Aug. 2008008	782	Cabbage	Copenhagen Mkt		Kenya Seeds	2003-4548	Kenya	?	1 x 10,000, 2 x 3,900	No
June' 2008	84	Cabbage	Copenhagen Mkt	Nyahururu	Simlaw	2004-6140-1	-	?	10,000	Yes
June' 2008	75	Cabbage	Copenhagen Mkt	Iten Nyahururu	Simlaw	2004-6140-2	Netherlands	?	10,000	No
June' 2008	90	Cabbage	Copenhagen Mkt	Nyeri	Simlaw	2004-6140-2	Netherlands	?	10,000	No
June' 2008	9	Cabbage	Copenhagen Mkt		Griffaton	345	France	Thiram	10,023	Yes
June' 2008	792	Cabbage	Copenhagen Mkt		Jumbo Agrovet (ex Royal Sluis)	634463	Netherlands	Thiram	1 x 10,000, 2 x 5,100	No
June' 2008	790	Cabbage	Copenhagen Mkt		Regina Seeds (Royal Sluis)	637509	Netherlands	Thiram	1 x 10,000, 2 x 4,800	No

June' 2008	44	Cabbage	Drum Head	Homa bay	Royal Sluis	852815	Netherlands	Thiram	10,000	No
June' 2008	34	Cabbage	Drum Head	Kisumu	Simlaw	2001-9691	-	?	10,000	No
June' 2008	26	Cabbage	F1	Kiligoris	Simlaw	02A4A	-	-	10,000	No
June' 2008	52	Cabbage	Gloria	Nakuru	Simlaw	62030	Denmark	Thiram	10,000	No
June' 2008	19	Cabbage	Gloria	Bomet	Simlaw	102030	Denmark	Thiram	10,000	No
June' 2008	24	Cabbage	Gloria	Mau-Narok	Kenya Seed	156455	Denmark	Thiram	10,000	No
June' 2008	783	Cabbage	Gloria		Kenya Seed	183960	Denmark	?	1 x 10,000, 2 x 3,000	No
June' 2008	42	Cabbage	Gloria	Kitale	Simlaw	187505	Denmark	Thiram	10,000	No
June' 2008	787	Cabbage	Gloria		E A Seeds	307079	Denmark	?	1 x 10,000, 2 x 3,100	No
June' 2008	45	Cabbage	Golden acre	Iten	Simlaw	2003-645	Denmark	Thiram	10,000	No
June' 2008	10	Cabbage	Kopkool	Migori	Kenya Seed	515001	-	-	10,000	No
June' 2008	79	Cabbage	Prize Drumhead	Kabarnet	E A Seeds	8194	-		10,000	No
June'. 2008	96	Cabbage	Prize Drumhead	Wote	Simlaw	50211		?	8,330	No
June' 2008	97	Cabbage	Pruktor F1	Kibwezi	Simlaw	167831	Denmark	Thiram	9905	No
June' 2008	100	Cabbage	Riana F1	Athi	Simlaw	137796	Denmark	Thiram	9450	No
June' 2008	82	Cabbage	Riana F1	Subukia	Simlaw	189231	Denmark	Thiram	10,000	No
June' 2008	102	Cabbage	Romenco	TZ Border	Regina Seeds (Royal Sluis)	491265	Netherlands	?	10080	No
June' 2008	60	Cabbage	Romenco	Nakuru	Regina	553703	Netherlands	?	10,000	No
June' 2008	80	Cabbage	Romenco Glory	Solai	Regina	933898	Netherlands	Thiram	10,000	No
June' 2008	15	Cabbage	Sugarloaf	Kehencha	Simlaw	502404	Kenya	-	10,000	No
June' 2008	118	Cabbage	Sugarloaf	Malindi	E A Seeds	905648	-	?Thiram	12,285	No
June' 2008	8	Cabbage	Sugarloaf	Migori	Kenya Seed	02-A44	-	TMTD	10,000	No
June' 2008	106	Cabbage	Sugarloaf	Taveta	Simlaw	2004-7176-1	-	?	11,935	No
June' 2008	83	Cabbage	Supermaster	Subukia	E A Seeds	185873	Denmark	Thiram	10,000	Yes
June' 2008	91	Cabbage	Victoria (Gloria)		Regina Seeds (Royal Sluis)	728225	Netherlands	Thiram	1 x 10,000, 2 x 2,360	No
June' 2008	93	Cabbage	Victoria (Gloria)		Jumbo Agrovet (ex Royal Sluis)	761474	Netherlands	Thiram	1 x 10,000, 2 x 4,500	No
June' 2008	81	Cauliflower	Kibo Giant	Subukia	Simlaw	182301	Denmark	?		No
July'2008	51	Chinese cabb.	Chihili	Nakuru	E A Seeds	22990	Arusha, TZ	?	10,000	No
July'2008	105	Chinese cabb.	Michihili	Taveta	Simlaw	2003-645-1	-	?	13,090	No

July'2008	116	Collards	Georgia	Malindi	Bonanaza Seeds	151001-926.3	USA	Thiram	11,585	No
July'2008	95	Collards	Georgia (Sukuma Wiki)		Jumbo Agrovet (ex E A Seeds)	02-4289 (022989)	Kenya	?	1 x 10,000, 2 x 4,000, 3 x 1,000	No
July'2008	789	Collards	Georgia (Sukuma Wiki)		E A Seeds	02-4289 (022989)	Kenya	?	1 x 10,000, 2 x 3,300	Yes
July'2008	20	Collards	Georgia (Sukuma Wiki)	Gucha	Simlaw	03-4836	USA	-	10,000	Yes
July'2008	6	Collards	Georgia (Sukuma Wiki)	Kehancha	Simlaw	03-4836	USA	-	10,000	Yes
July'2008	32	Collards	Georgia (Sukuma Wiki)	Sondu	E A Seeds	03-4836-1	USA	?	10,000	Yes
July'2008	36	Collards	Georgia (Sukuma Wiki)	Vihiga	E A Seeds	03-4836-1/303040	USA	?	10,000	No
July'2008	111	Collards	Georgia (Sukuma Wiki)	Kongawea	E A Seeds	03-4836-1/303040	USA	?Thiram	8,575	No
July'2008	66	Collards	Georgia (Sukuma Wiki)	Kapkoi/Marigat	E A Seeds	04-7430/406057	USA	?	10,000	No
July'2008	95	Collards	Georgia (Sukuma Wiki)	Machatos	E A Seeds	04-7430/406057	USA	?	9,450	No
July'2008	91	Collards	Georgia (Sukuma Wiki)	Nanyuki	E A Seeds	04-7430/406057	USA	?	10,000	No
July'2008	120	Collards	Georgia (Sukuma Wiki)	Mombassa	E A Seeds	04-7430/406057	USA	?	9,975	No
July'2008	41	Collards	Sukuma Wiki	Butere	E A Seeds	21299	USA	?	10,000	No
July'2008	55	Collards	Sukuma Wiki	Nakuru	Simlaw	22083	Kenya	?	10,000	No
July'2008	5	Collards	Sukuma Wiki	Isebania	E A Seeds	02-4283	USA	-	10,000	No
July'2008	11	Collards	Sukuma Wiki	Isebania	E A Seeds	02-4283	USA	-	10,000	No
July'2008	85	Collards	Sukuma Wiki		Kenya Seed	02-5025	Kenya	?	1 x 10,000, 2 x 4,000	No
July'2008	17	Collards	Sukuma Wiki	Kehencha	Simlaw	02-5025	Kenya	-	10,000	No
July'2008	9	Collards	Sukuma Wiki	Migori	E A Seeds	04-5050	-	-	10,000	No
July'2008	4	Collards	Sukuma Wiki	Awendo	Simlaw	04-5051	Denmark	Thiram	10,000	No
July'2008	64	Collards	Sukuma Wiki	Timborua/Iten	Simlaw	04-5051	-	?	10,000	Yes
July'2008	89	Collards	Sukuma Wiki	Naro-moru	Simlaw	04-5051	-	?	10,000	Yes
July'2008	115	Collards	Sukuma Wiki	Mtwapa	Simlaw	04-5051	-	?	10,000	
July'2008	115	Collards	Sukuma Wiki	Mtwapa	Simlaw	04-5051	-	?	8,225	No
July'2008	101	Collards	Sukuma Wiki	Kibwezi	Simlaw	04-5051	-	?	7,245	No
July'2008	93	Collards	Sukuma Wiki	?	Simlaw	04-5068	-	?Thiram	7,315	No
July'2008	97	Kale	Kinale	Wangigi	Market	none	Kenya	none	1 x 10,000, 2 x 4,400	No
July'2008	31	Kale	Kinale	Nakuru	Market	none	Kenya	-	10,000	No
July'2	96	Kale	Komolo	Wangigi	Market	none	Kenya	none	1 x	No

008									10,000, 2 x 4,800	
July'2008	43	Kale	Sukuma Wiki	Luanda	Farmer	none	Kenya	?	10,000	No
July'2008	13	Kale	Sukuma Wiki	Nakuru	Market	none	Kinale	-	9,906	No
July'2008	7	Kale	Th. Headed	Migori	Service Plus	3	France	TMTD	10,000	No
July'2008	18	Kale	Th. Headed	Bomet	Griffaton	3	France	TMTD	10,000	No
July'2008	13	Kale	Th. Headed	Isebania	E A Seeds	3	-	-	10,000	Yes
July'2008	61	Kale	Th. Headed	Nakuru	Griffaton	3	France	TMTD	10,000	Yes
July'2008	88	Kale	Th. Headed	Karatina	Griffaton	3	France	TMTD	10,000	Yes
July'2008	110	Kale	Th. Headed	Kongowea	Vet & Agron. Ltd (ex Griffaton)	03	France	TMTD	10,150	No
July'2008	21	Kale	Th. Headed	Narok	Griffaton	24	France	TMTD	10,000	No
July'2008	38	Kale	Th. Headed	Siaya	Simlaw	02-A5025	-	?	10,000	No
July'2008	23	Kale	Th. Headed	Narok	E A Seeds	03-4835	-	-	10,000	No
July'2008	40	Kale	Th. Headed	Capenguria	E A Seeds	03-4835	-		10,000	No
July'2008	1	Kale	Th. Headed	Kericho	E A Seeds	03-4835	Denmark	Thiram	10,000	Yes
July'2008	88	Kale	Th. Headed		E A Seeds	03-4835 (303038)	USA	?	1 x 10,000, 2 x 4,770	No
July'2008	94	Kale	Th. Headed		Jumbo Agrovet (ex E.A. Seeds)	03-4835 (303038)	USA	?	1 x 10,000, 2 x 4,800	No
July'2008	77	Kale	Th. Headed	Muserechi	E A Seeds	03-4835 (303038)	-	?	10,000	Yes
July'2008	84	Kale	Th. Headed		Kenya Seed	2003-405	Denmark	?	1 x 10,000, 3 x 1,000	Yes
July'2008	12	Kale	Th. Headed		Kenya Seed	2003-605	Denmark	Thiram	10,012	No
July'2008	87	Kale	Th. Headed	Olkalau	Simlaw	2004-6140-2	-	?	10,000	Yes
July'2008	121	Kale	Th. Headed	Mombassa	Simlaw	2004-6140-7	-	?	9,590	Yes
July'2008	27	Kale	Unknown	Migori	Farmer	none	-	-	10,000	No
July'2008	37	Kale	Unknown	Mumias	Farmer	none	Kenya	?	10,000	No
July'2008	28	Kale	Unknown	Kipisorwet	Farmer	none	Kenya	-	10,000	Yes
July'2008	29	Kale	Unknown	Keumbo	Market	none	Kenya	-	10,000	Yes
July'2008	50	Kale	Unknown	Nakuru	Market	none	Kenya	-	10,000	Yes
July'2008	67	Kale	Unknown	-	Market	none	Kenya	-	10,000	Yes
July'2008	30	Kale?	Unknown	Keumbo	Market	none	Kenya	-	10,000	Yes
July'2008	11	Rape seed	Unknown	Njoro	Market	none	-	-	9,729	Yes

Appendix III: Infected Seeds with Xcc

Table VI.

	No.	Type	Cultivar	Collection centre	Supplier	Lot No.	Origin	Treat	Packed	No tested	Xcc detected
June'2008	84	Cabbage	Copenhagen Mkt	Nyahururu	Simlaw	2004-6140-1	-	?		10,000	Yes
June'2008	9	Cabbage	Copenhagen Mkt		Griffaton	345	France	Thiram		10,023	Yes
June'2008	83	Cabbage	Supermaster	Subukia	E A Seeds	185873	Denmark	Thiram		10,000	Yes
July'2008	89	Collards	Georgia (Sukuma Wiki)		E A Seeds	02-4289 (022989)	Kenya	?		1 x 10,000, 2 x 3,300	Yes
July'2008	20	Collards	Georgia (Sukuma Wiki)	Gucha	Simlaw	03-4836	USA	-		10,000	Yes
July'2008	6	Collards	Georgia (Sukuma Wiki)	Kehancha	Simlaw	03-4836	USA	-		10,000	Yes
July'2008	32	Collards	Georgia (Sukuma Wiki)	Sondu	E A Seeds	03-4836-1	USA	?		10,000	Yes
July'2008	89	Collards	Sukuma Wiki	Naro-moru	Simlaw	04-5051	-	?		10,000	Yes
July'2008	64	Collards	Sukuma Wiki	Timborua/ Iten	Simlaw	04-5051	-	?		10,000	Yes
July'2008	13	Kale	Th. Headed	Isebania	E A Seeds	3	-	-		10,000	Yes
July'2008	61	Kale	Th. Headed	Nakuru	Griffaton	3	France	TMTD		10,000	Yes
July'2008	88	Kale	Th. Headed	Karatina	Griffaton	3	France	TMTD		10,000	Yes
July'2008	1	Kale	Th. Headed	Kericho	E A Seeds	03-4835	Denmark	Thiram		10,000	Yes
July'2008	77	Kale	Th. Headed	Muserechi	E A Seeds	03-4835 (303038)	-	?		10,000	Yes
July'2008	121	Kale	Th. Headed	Mombassa	Simlaw	2004-6140-7	-	?	Sep-04	9,590	Yes
July'2008	84	Kale	Th. Headed		Kenya Seed	2003-405	Denmark	?		1 x 10,000, 3 x 1,000	Yes
July'2008	87	Kale	Th. Headed	Olkalau	Simlaw	2004-6140-2	-	?		10,000	Yes
July'2008	28	Kale	Unknown	Kipisorwet	Farmer	none	Kenya	-		10,000	Yes
July'2008	11	Rape seed	Unknown	Njoro	Market	none	-	-		9,729	Yes
July'2008	50	Kale	Unknown	Nakuru	Market	none	Kenya	-		10,000	Yes
July'2008	67	Kale	Unknown	-	Market	none	Kenya	-		10,000	Yes
July'2008	30	Kale?	Unknown	Keumbo	Market	none	Kenya	-		10,000	Yes
July'2008	29	Kale	Unknown	Keumbo	Market	none	Kenya	-		10,000	Yes

Appendix IV: Determination of Race Type

Table VII

Sample No.	Type	Cultivar	Collection centre	Supplier	Lot No.	Origin	Treat	Xcc detected	Savoy cabbage	Race type
84	Cabbage	Copenhagen Mkt	Nyahururu	Simlaw	2004-6140-1	-	?	Yes	+ve	3
9	Cabbage	Copenhagen Mkt		Griffaton	345	France	Thiram	Yes	+ve	3
83	Cabbage	Supermaster	Subukia	E A Seeds	185873	Denmark	Thiram	Yes	+ve	1
89	Collards	Georgia (Sukuma Wiki)		E A Seeds	02-4289 (022989)	Kenya	?	Yes	+ve	1
20	Collards	Georgia (Sukuma Wiki)	Gucha	Simlaw	03-4836	USA	-	Yes	+ve	1
6	Collards	Georgia (Sukuma Wiki)	Kehancha	Simlaw	03-4836	USA	-	Yes	+ve	1
32	Collards	Georgia (Sukuma Wiki)	Sondu	E A Seeds	03-4836-1	USA	?	Yes	+ve	1
89	Collards	Sukuma Wiki	Naro-moru	Simlaw	04-5051	-	?	Yes	+ve	1
64	Collards	Sukuma Wiki	Timborua/Iten	Simlaw	04-5051	-	?	Yes	+ve	1
13	Kale	Th. Headed	Isebania	E A Seeds	3	-	-	Yes	+ve	1
61	Kale	Th. Headed	Nakuru	Griffaton	3	France	TMTD	Yes	+ve	1
88	Kale	Th. Headed	Karatina	Griffaton	3	France	TMTD	Yes	+ve	3
1	Kale	Th. Headed	Kericho	E A Seeds	03-4835	Denmark	Thiram	Yes	+ve	3
77	Kale	Th. Headed	Muserechi	E A Seeds	03-4835 (303038)	-	?	Yes	+ve	
121	Kale	Th. Headed	Mombassa	Simlaw	2004-6140-	-	?	Yes	+ve	3
84	Kale	Th. Headed		Kenya Seed	2003-405	Denmark	?	Yes	+ve	1
87	**Kale**	**Th. Headed**	**Olkalau**	**Simlaw**	**2004-6140-2**	**-**	**?**	**Yes**	+ve	**1**
28	Kale	Unknown	Kipisorwet	Farmer	none	Kenya	-	Yes	+ve	1
11	Rape seed	Unknown	Njoro	Market	none	-	-	Yes	+ve	1
50	Kale	Unknown	Nakuru	Market	none	Kenya	-	Yes	+ve	1
67	Kale	Unknown	-	Market	none	Kenya	-	Yes	+ve	1
30	Kale?	Unknown	Keumbo	Market	none	Kenya	-	Yes	+ve	1
29	Kale	Unknown	Keumbo	Market	none	Kenya	-	Yes	+ve	1

All the points inoculated Xcc isolates showed the typical symptoms and was easy to raece typee in all the isolates.

Appendix V: Region that Recorded the Highest Incidence of Xcc

Table 7 Region recorded the highest incidence of Xcc

Collection centre		Xcc detected		Total
		Not present	Present	
Athi	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Awendo	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Baitany	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Bomet	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Busia	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Butere	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Gucha	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Homa bay	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Isebania	Count	3	0	3
	% of Total	2.7%	0.0%	2.7%
Iten	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Iten/Nyahururu	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kabarnet	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kapenguria	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kapkoi/Marigat	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Karatina	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kehancha	Count	4	0	4
	% of Total	3.6%	0.0%	3.6%
Kericho	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Keumbo	Count	0	2	2
	% of Total	0.0%	1.8%	1.8%
Kibwezi	Count	1	1	2
	% of Total	0.9%	0.9%	1.8%
Kiligoris	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kipisorwet	Count	0	1	1
	% of Total	0.0%	0.9%	0.9%
Kisumu	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Kitale	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%

Kongawea	Count	3	0	3
	% of Total	2.7%	0.0%	2.7%
Luanda	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Machackos	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Machatos	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Malaba	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Malindi	Count	3	0	3
	% of Total	2.7%	0.0%	2.7%
Matuu	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Mau-Narok	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Migori	Count	5	1	6
	% of Total	4.5%	0.9%	5.5%
Moi's Bridge	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Mombassa	Count	1	1	2
	% of Total	0.9%	0.9%	1.8%
Mtwapa	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Mumias	Count	1	1	2
	% of Total	0.9%	0.9%	1.8%
Muserechi	Count	0	1	1
	% of Total	0.0%	0.9%	0.9%
Nakuru	Count	8	1	9
	% of Total	7.3%	0.9%	8.2%
Nanyuki	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Naro-moru	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Narok	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Njoro	Count	0	1	1
	% of Total	0.0%	0.9%	0.9%
Nyahururu	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Nyeri	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Olkalau	Count	0	1	1
	% of Total	0.0%	0.9%	0.9%
Siaya	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Solai	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Sondu	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Subukia	Count	3	0	3
	% of Total	2.7%	0.0%	2.7%
Taveta	Count	2	0	2

	% of Total	1.8%	0.0%	1.8%
Timboroa/	Count	1	0	1
Iten	% of Total	0.9%	0.0%	0.9%
TZ Border	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Unknown	Count	11	7	18
	% of Total	10.0%	6.4%	16.4%
Vihiga	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Wangigi	Count	2	0	2
	% of Total	1.8%	0.0%	1.8%
Wote	Count	1	0	1
	% of Total	0.9%	0.0%	0.9%
Total	**Count**	**92**	**18**	**110**
	% of Total	**83.6%**	**16.4%**	**100.0%**