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A Review of Diseases Found in Cabbage and the Effects of Ultraviolet -B on Black Rot.

Dameion J. Crook

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THESIS

BY

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A REVIEW OF DISEASES FOUND IN CABBAGE

AND

THE EFFECTS OF ULTRAVIOLET-B ON BLACK ROT

By

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The objective of this study is to determine the effects of elevated UV-B (320 - 290 nm) light on Xanthomonas campestris. Xanthomonas campestris is a bacterium that causes a disease commonly known as Black Rot. This disease affects crucifers a large variety of vegetable crops. The disease is easily recognized by the presence of yellow, V-shaped lesions extending inward from the margin of the leaf. The disease progresses, the yellow lesions turn brown and the tissue dies. Veins darken and the mid-rib of leaves turn black within in the infected area. The veins discoloration progresses toward the base of the leaf as the bacteria spread through the leaf veins. Eventually, the bacterium spreads into the main stem. In this study cabbage plants infected with Xanthomonas campestris will be exposed to low doses of UV-B (320 - 290 nm) for short periods of time in a greenhouse. To test the effects on Xanthomonas Campestris two groups of cabbage inoculated with Xanthomonas campestris will be treated with a UV-B (320 - 290 nm) radiation. The treatments will one hour daily for a two-week period, with both groups receiving the same amount of natural light and irrigation.

Nursery and greenhouse crops are the sixth largest agricultural commodity group in the U. S. with a farm gate value $12.1 billion in 1998. The greenhouse crop industry annual

growth rate in Texas is 9.3% and in 2001 the industry contributed 1.1 billion dollars to the Texas state economy. This growth is due to increasing urbanization and rapid growth of new residential and commercial developments. Greenhouses also present an environment in which elements such as temperature and sunlight that can be controlled by farmers. Despite the rapid develop of this industry and its positive economic impact; the greenhouse crop industry faces many obstacles. A major challenge to farmers who grow crops by this alternative method is the constant threat of disease. In a 5-year period, 15% of cabbage fields will be affected with black rot. By developing methods that would otherwise eliminate disease-causing bacteria, it would increase crop production and decrease crops loss.

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CHAPTER 1

INTRODUCTION

Background

The Earth's surface is receiving increased amounts of Ultraviolet (UV)-B (290 - 320 nm) due to depletion of the ozone layer. UV-B can be absorbed by DNA and produces specific chemical changes that disrupt the structure of the DNA. This can cause death to the cell or lead to mutations (changes in DNA sequences). In humans exposure to UV-B radiation is associated with increased skin cancer rates. Other organisms can be adversely affected by exposure to UV-B. They may be more sensitive to damage from the radiation at different times in their life cycle due to changes in growth rates and differences in protective coverings or pigmentation. Ultraviolet, (UV) radiation can be absorbed by molecules in the cell producing specific chemical changes (Bornman, 1989). The most common chemical change produced when DNA absorbs UV is the production of a covalent bond between adjacent pyrimidines resulting in a pyrimidine dimmer. These dimers disrupt the replication of DNA (Bornman, 1989). Ultraviolet radiation is divided into 3 ranges, UV-A (400 - 360nm), UV-B (320-290 nm), and UV-C (290 - 180 nm).

Agricultural scientists have responded with a series of pioneering investigations on the effect of artificial and solar UV radiation upon plant growth

and development. A great variety of physiological and morphological plant responses to UV radiation have been subsequently demonstrated over the past years. Most of these experiments, however, have employed UV lamps, which usually emit radiation quite unlike the radiation present in the normal terrestrial solar spectrum. The importance of solar angle, atmospheric turbidity, elevation above the sea level, cloud cover, total atmospheric ozone column, and the UV albedo of the earth's surface with respect to the total UV irradiation intensity and wavelength composition should be considered in UV radiation of natural environments. Though not all the plant responses demonstrated as the result of UV radiation are considered as damaging or disadvantageous for the plant, the majority of evidence indicates that UV irradiation is usually detrimental, particularly UV-B irradiation (Caldwell, 1999).

Recent Developments

The enhanced UV-B radiation generally has negative impacts on growth, yield and quality of some crop plants such as soybean, winter wheat, rice, sorghum, cotton and corn. The response varies with different plant species. Some are very sensitive and some are less sensitive. With enhanced UV-B radiation, photosynthesis decreases, plant height and leaf area decrease, dry matter production, yield and quality are reduced in many crops. In the study conducted by Tevini et al. (1991), plant height, leaf area, and the dry weight of sunflower, corn, and rye seedlings were significantly reduced with enhanced UV-B radiation. Rice is among the most important crop plants in the world. Sixteen rice cultivars from several different geographical regions were grown in greenhouses with

supplemental levels of UV-B radiation (Teramura et al., 1991). Alterations in biomass, morphology, and, maximum photosynthesis were determined. Approximately one-third of all cultivars tested showed a statistically significant decrease in total biomass with increased UV-B radiation. For these sensitive cultivars, leaf area and tiller number were also significantly reduced. Photosynthetic capacity, as determined by oxygen evolution, declined for some cultivars. In a six-year field study of a UV-sensitive soybean, Teramura et al. (1991) presented a statistically significant 19%-25% reduction in seed yield in five of the six years under a 25% ozone reduction level.

Research Objectives

This study satisfies two purposes; the first is a review of disease found in cabbage and the second study is to determine the effects of administering low doses of Ultraviolet-B (UV-B) on *Xanthomonas campestris* (Xcc) the bacteruim commonly known as Black rot in cabbage. By determining the effects of low dose of UV-B as a treatment, it is possible to minimize the loss of crucifers due to this plant disease. There are many environmental factors that affect disease severity and the plant's ability to resist disease: they include light, temperature, humidity and nutrition. Some studies indicate that UV-B radiation can increase or decrease disease severity depending on the host-pathogen combination (Teramura, 1990). Lambe et al., (1982) found a reduction in disease severity of bean and oat rust and wheat powder mildew with UV-B radiation in growth chambers studies. However, Biggs et al. (1981) recorded a significant increase in severity of a natural rust epidemic caused by *Puccinia recondite* with increasing

UV-B irradiation in susceptible wheat cultivars. It is clear that the effects of UV-B irradiation on the host-parasite interaction are complex and highly variable. This study examines and describes what effects of UV-B irradiation may on Black Rot found in cabbage.

CHAPTER 2

REVIEW OF LITERATURE

History of Cole Crops

Cole crops are in the Brassicaceae family, formerly called Cruciferae, and many are in the species Brassica oleracea. A kale-like ancestor was grown in gardens as far back as the time of the Roman Empire. In Europe, cabbage gardens were very important food sources during the Middle Ages. Differences in morphology between cole crops are undoubtedly the result of early selection for various edible parts. This selection was made easier by the reproductive biology of cole crops.

All cole crops are interfertile (i.e., they can be crossed) and many are self-incompatible (i.e., flowers cannot be fertilized by pollen from the same plant). These characteristics have made it easy to select for new types of cole crops. Self-incompatibility also makes hybrid seed production economical (Pierce, 1987) Because of their vigorous growth, uniformity of maturation and disease resistances, hybrids are preferred by most growers over the older open-pollinated cultivars.

Cole Crop Characteristics

Cole crops grow best in cool weather. In Alaska, cabbages can grow to 68 pounds with 5-foot diameters. Cabbage plants can continue to grow at temperatures as low as 41 degrees F but little growth occurs over 77 degrees F. Cabbage, kale, collards and kohlrabi are biennials, flowering only after a period of prolonged cold weather. Cauliflower and broccoli have been selected to bloom as annuals and require less chilling to flower, but they will head sooner in cold weather.

Cabbage stems are non-branched and grow upward very little before expanding in diameter without further growth in length. The first leaves unfold normally to form what is called the frame. Leaves produced later only unfold partially. The inner leaves are then enclosed and can't expand. They will form a solid head as they develop, but if the plants grow too rapidly, or produce a flower stalk, the pressure of the inner leaves against the outer can split the head wide open. The plant parts eaten differ between the various cole crops more than in any other group of vegetables. In kale and cabbage, leaves are eaten; in broccoli, the green buds and fleshy flower stalks; in cauliflower, the thick flower stalks making up the round, white head or curd; in Brussels sprouts, the axillary bud; in kohlrabi: the swollen leaf base.

Location of Production

The cole crops of cabbage, kale, collards, turnips, rutabaga, Brussels sprouts, broccoli and cauliflower, are among the most widely grown vegetables in the temperate zone (Konsler, 1981). Cole crops are also widely grown during the

cool season in tropical and subtropical areas. In 1999, 40 percent of the frozen broccoli and 46 percent of the frozen cauliflower consumed in the United States was imported. The top cabbage-producing states in 2002 by acreage were New York, Texas, California, Wisconsin, and Florida. The top broccoli producing states in 2002

were California, Arizona, and Texas. Top states in cauliflower production were California, Arizona, Oregon, and New York (U.S. Dept. of Commerce and Bureau of the Census 2002).

Production Practices of Cabbage

Soils and Fertilization

Cabbage is grown on all soil types, but does best on well-drained sandy loams with high organic matter. A pH of 6 to 6.5 is optimal, although cabbage is sometimes grown at higher pH (pH > 7.2) for clubroot control. Lower pH values will reduce growth. Soil calcium levels should be 1000 to 2000 ppm and magnesium 150 to 300 ppm. (Parnes, 1990). Any lime needed should be added well ahead of planting. Soil Management describes types of liming materials and their effect on soil pH, and Ca and Mg availability. Low calcium in the soil can cause tipburn, but this physiological disorder can also occur if calcium is unavailable because of drought or flooded soil, or when soil fertility is high..

Like most leafy vegetables, cabbage has a high nitrogen requirement. Too little nitrogen reduces yields, shortens storage life, delays maturity, and can increase the 'cabbagey' flavor to objectionable levels. Too rapid growth at high nitrogen, however, is likely to lead to coarse, loose heads, cracking, tipburn, and

poor processing and storage quality. Red cabbage needs an additional 10 lb actual nitrogen per acre per side-dressing. This nitrogen requirement can be satisfied by a wide variety of soil amendments, as well as conventional fertilizers.

Harvesting one ton of cabbage removes 4 to 9 pounds N, 1 to 4 pounds P2O5, and .8 to 1.3 pounds K2O from the soil. Fertilizer recommendations for cabbage based on soil tests are presented in Cole crop nutrient recommendations based on soil tests. Organic sources of these nutrients are listed in Soil Management. Typically, if soil test results indicate a need for high analysis N-P-K, half the amount should be broadcast and half banded to reduce the potential for salt injury to roots from the banded material. Preplant applications are followed three weeks after transplanting by a side dressing of 25 to 30 pounds of nitrogen. Cole crops have a high boron requirement. Symptoms of boron deficiency vary with the cole crop attacked. Cabbage heads may simply be small and yellow. Most cole crops develop cracked and corky stems, petioles and midribs. The stems of broccoli, cabbage and cauliflower can be hollow and are sometimes discolored. Cauliflower curds become brown and leaves may roll and curl. If boron is added, and beans or other boron sensitive crops follow cabbage in a rotation, a soil test is advisable before planting to ensure that boron levels are not too high.

Planting

Cover crops and conservation tillage are often used with cabbage, especially in hilly areas where the potential for erosion is high. Cabbage is usually transplanted in no-till situations to increase early growth. With conventional

tillage practices, seeds can be planted directly in the ground or the crop can be established using transplants. In the field, seeds are placed 1 to 2 inches apart in rows 36 to 42 inches apart, 3- to 4,-inch deep and are thinned to 9 to 14 inches apart. This spacing will produce a high percentage of 2-to-3-pound heads which is preferred for fresh market production. Using double rows per bed (or streaks per row) will increase yields 50 to 70 percent and improve harvest efficiency. With double streaks, spacing must be changed to 14 to 18 inches and plants must be staggered in the row, with 12 inches between streaks.

Direct seeding is most common for fall crops when the soil is warm and emergence and early growth is rapid. The spring crop is usually started from transplants, using seedling final-stand spacings. Transplants can be overwintered but if seedlings larger than 1 inch are exposed to low temperatures (32 to 45 degrees F for extended periods), flowering can be induced, which results in bolting rather than production of a head. If not overwintered, transplants are typically set out three weeks before the last frost date. Optimal transplant size for either fall or spring planting is 4 to 6 true leaves. Deep planting has been shown to increase yields in once-over harvested cabbage.

Harvest and Storage

In Texas, 85 to 90 days from transplanting to harvest is typical. Heads should be firm-to-hard at harvest, but delaying harvest may increase the risk of splitting mature heads if soil moisture increases suddenly. Two-to-three pound heads are cut at the base and the outer leaves are trimmed off. For the fresh market, fields may be cut 3 to 5 times. Hybrids are preferred for commercial

production because a higher percentage of the plants can be harvested at any one time, thus reducing the total number of harvests. Cabbage is packed in wirebound containers or sacks weighing approximately 50 pounds.

Heads must be cooled immediately after harvest. Cabbage can be stored at 32 to 36 degrees F and 95 percent relative humidity for 3 to 6 weeks (early crop) or 5 to 6 months (late crop). Storage life can be prolonged even further at low O2 (2 percent) and high CO2 (5 percent) and with controlled atmosphere storage systems. Bacterial soft rot is the main problem in storage. Since fresh cabbage is available almost year-round, for the most part, only special cultivars used for sauerkraut processing are stored.

Primary Diseases of Cabbage

Alternaria leafspot (Alternaria spp.)

Symptoms of Alternaria leafspot include circular lesions with sunken centers surrounded by bright yellow chlorotic halos with ragged margins. These lesions first form on older leaves and gradually turn dark brown. Cool, wet weather favors disease development. Alternaria leafspot also infects Brussels sprouts and causes brown rot of cauliflower. Alternaria can be transmitted on the seed and in plant debris.

Blackleg (Leptosphaeria maculens)

This fungal disease of cole crops does not usually cause losses in the field unless it is introduced by using infected seed. The first symptoms of this fungal disease are spots on the leaves and a depressed light brown canker near the base of the stem. Numerous black dots form on the canker and on the leaves. The stem

canker spreads down to the roots, which are eventually killed, stunting the plant. With high rainfall, the disease can spread regardless of temperature, although it is more active at cooler temperatures. Blackleg can also infect the crop in storage. Blackleg will not survive on seed that is soaked in 122 degrees F water for 25 minutes. Unfortunately, hot water treatment reduces germination so seed should be tested before planting. Growers can purchase seed tested (indexed) for low incidence of this pathogen. Infected transplants are also often sources of blackleg. Methods of reducing infection from transplants include:

1. rotating transplant beds out of crucifer production for at least 4 years,
2. ensuring good air drainage and rapid evaporation of dew,
3. avoiding spraying or dipping transplants in water,
4. using new containers for shipping transplants.

Sheep manure can be a source of blackleg inoculum if the sheep have grazed on infected plants. Blackleg symptoms are worse on plants also damaged by herbicide.

Clubroot (Plasmodiophora brassicae)

Clubroot is not a serious problem in most of the south because it is favored by cool, wet soils. Symptoms of clubroot on cole crops are an enlarged or club-bed root system and a wilted or stunted shoot. Younger plants can be killed and older plants weakened to the extent that they will not produce a marketable crop. The fungus can be introduced on transplants or by irrigation from ponds receiving drainage water from infected fields. Because clubroot is so long-lived, a 7-to-10-year rotation out of crucifers is required for control. There are relatively few other

control options. Raising soil pH to 7.2 by broadcasting and incorporating hydrated lime into the soil at a rate of 1500 pounds per acre 2 or 3 days before transplanting offers some control. This limiting should not be done more than once every 3 years to keep the soil from becoming too alkaline, nor is it very effective on light, sandy or loose muck soils. Clubroot-resist plant cultivars of kale, turnip and rutabaga are commercially available. Experiments in Australia compared fumigation and solarization and a combination of the two for efficacy in controlling clubroot. Plastic sheeting for solarization was installed at the time of methyl bromide fumigation, and within 4 hours of dazomet application. For all solarized plots, plastic was left in place for 7 weeks. In clay soil, solarization combined with low rates (90 pounds per acre compared to the standard rate of 670 pounds per acre) of fumigants (dazomet and methyl bromide) controlled club root in cauliflower better than either fumigant alone at low or high rates (90 or 670 pounds per acre) or solarization alone. On sandy soils, however, the fumigants alone gave the same control as solarization plus fumigant.

Downy Mildew (Peronospora parasitica)

Downy mildew is of minor importance in the field, but can become systemic (move through the whole plant) and kill seedlings in seedbeds. This fungal disease of cole crops causes upper leaf surfaces to become chlorotic with black spots, while diffuse white fungal growth is found on the lower surfaces. Spread is most rapid during rainy periods and at temperatures of 50 to 60 degrees F. Two-year rotations and clean, well-drained soils can lower disease potential.

Fusarium yellows (Fusarium oxysporum f. conglutinans)

This fungal disease of cole crops results in an uneven yellowing of plants progressing upward from the lower leaves. It is most severe when soil temperatures are above 70 degrees F, and least severe below 60 degrees F. The yellows pathogen persists in the soil for many years, but Fusarium yellows is effectively controlled by use of resistant cultivars.

Black Rot (Xanthomonas campestris)

Black rot is caused by a bacterium to which all brassicas are susceptible. This bacterium is seed-borne, but can also over-winter on weeds and crop debris. Worldwide, black rot is considered to be the most important disease of crucifers. Infected leaves develop a wedge-shaped yellowing on the margins, often followed by blackening of the veins and dying of V-shaped sections of the leaves. Under warm, humid conditions, symptoms appear 10 to 14 days after infection. Black rot may not appear when temperatures are low, but spreads rapidly during periods of rain and high temperatures.

The following practices are suggested as controls for black rot:

1. three-year rotation of the seedbed and field,
2. hot water disinfection of the seeds (122 degrees F for 25-30 minutes),
3. using black rot tolerant or resistant cultivars
4. using certified disease-free transplants.

In most states, it is possible to purchase state inspected and certified black rot-free transplants. Practices such as topping transplants to toughen them, and dipping or spraying transplants with water after digging, spread black rot and

should be avoided. Controlling cruciferous weeds and using only new containers for shipping transplants will also reduce black rot incidence. Chemical control of black rot involves the use of a fixed copper fungicide to prevent disease spread.

An Expanded Perspective of Black Rot

Black rot is caused by a bacterium, Xanthomonas campestris pv. campestris, that can infect most crucifer crops at any growth stage. This disease is difficult for growers to manage and is considered the most serious disease of crucifer crops worldwide (Figure 1). The disease can cause significant yield losses when warm, humid conditions follow periods of rainy weather during early crop development. Late infections can provide a wound for other rot organisms to enter and cause significant damage during storage.

Symptoms of Black Rot

Symptoms of black rot vary considerably depending on the host, cultivar, plant age and environmental conditions. The bacteria can enter plants through natural openings and wounds caused by mechanical injury on roots and leaves. Seed-borne bacteria infect the emerging seedlings through pores on the margin of the cotyledons and then spread systemically through the seedling. Infected seedlings grown in the greenhouse under cool conditions (below 15–18°C) frequently do not show any symptoms of the disease. When infected seedlings are transplanted to the field and temperatures rise to 25–35°C during periods of high relative humidity (80–100%), they become stunted with dead spots on the cotyledons (Figure 2) and will eventually wilt, and die. In regions with temperate

Figure 1. Mature Cabbage Plant

The classic symptom of black rot is caused by local infection that results when bacteria enter leaves through hydathodes, which are natural openings at leaf margins. The infected tissue is wilted and pale green initially becomes yellow, then turns brown and dies. Affected areas are usually wedge or V-shaped when bacteria enter leaves through hydathodes.

Figure 2. Young Cabbage Plant

Plants can be infected during any growth stage. Two types of symptoms occur depending on whether infection is systemic or local. Seedlings that are infected systemically become yellow, drop lower leaves, and may die

climates (where temperatures remain cool), disease symptoms on infected seedlings may not always be obvious or appear severe. Infected seedlings grown under cool conditions may ooze bacteria from pores and lesions, which then serve as a source of the pathogen for neighboring plants. On older plants, the disease symptoms often appear as yellow or dead tissue at the edges of leaves, similar to tip burn, except the lesion frequently progress into a V-shape with the base of the V usually directed along a vein (Figure 3). Close inspection of infected leaves and stems may reveal black veins running through the infected tissue from which the disease gets its name (Figure 4). Lesions on leaves can expand down toward the base of the leaf causing the leaf to wilt and die.

The bacteria produce a sticky polysaccharide called xanthan that eventually plugs the vascular tissue inside the veins causing them to collapse and turn black. The tissue above the plugged, collapsed xylem eventually turns yellow, wilts and dies. During hot humid environmental conditions, the bacteria can move from the leaf into the stem through the xylem. Once inside the stem, the bacteria can move up or down to other parts of the plant including the roots. (Figure 5.) Systemically infected plants may produce chlorotic areas anywhere on the leaf. Severely infected leafy cole crops such as kale and cauliflower tend to shed their leaves from the bottom up leaving only a tuft of distorted leaves separated from the root system by a scarred barren stem. Symptoms on cauliflower often appear as black flecks or scorched leaf margins. The curds of infected cauliflower heads often become blackened.

Figure 3. Cabbage Leaf Close Up.

Close inspection reveals black veins through infected tissue. Plants infected systemically because of contaminated seed may not develop symptoms for many weeks. These areas enlarge as the disease progresses, and severely affected leaves may drop off. Bacteria also can enter leaves through wounds, including those made by insects.

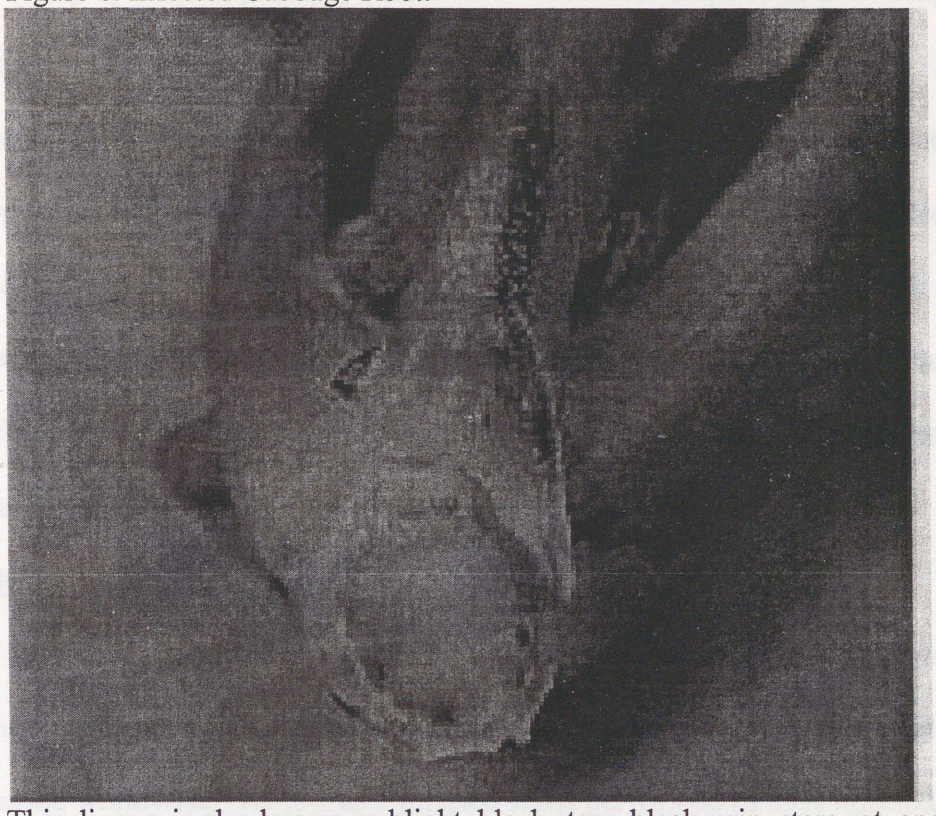
Figure 4. Early Stages of Black Rot

(Early stages of Black rot)
Yellow v-shaped spots found on a mature cabbage plant.

Figure 5. Cabbage Leaf Close Up

The veins in infected leaves, stems, and roots sometimes become black because the bacteria produce an extra-cellular polysaccharide that plugs normal water flow and blackened veins can be seen in stems and leaf petioles by cutting crosswise.

Figure 6. Infected Cabbage Root.



This disease is also known as blight, black stem, black vein, stem rot, and stump rot. Once inside the stem, the bacteria can move up or down to other parts of the plant including the roots.

Foliar symptoms may not be visible on infected root crops such as rutabaga and radish but blackened vascular tissue can appear inside the edible root tissue rendering the plants unmarketable. Although some infected plants may appear healthy, cutting across infected stems will reveal characteristic blackened vascular tissue. This is a simple method of determining the presence of the disease. Some symptoms of black rot closely resemble those caused by Fusarium yellows, which causes the vascular tissue to turn brown. Most commercial crucifer cultivars are resistant to Fusarium.

The Spread of Black Rot

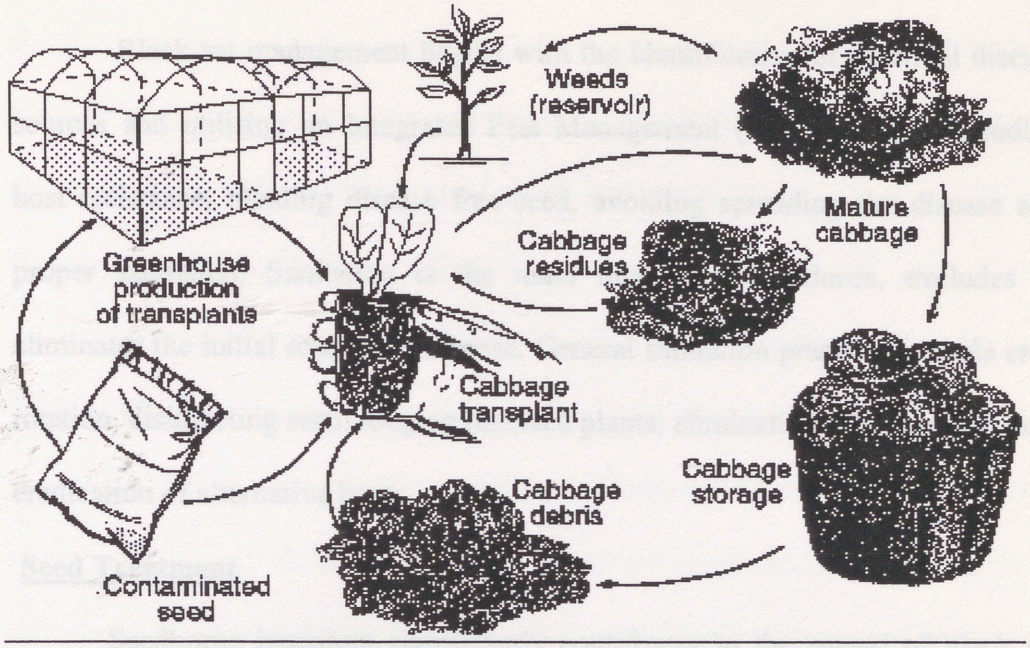
Seed contaminated with black rot bacteria is considered the most important source of the pathogen and significantly contributes to the spread of this disease worldwide. As few as 3 infected seeds per 10,000 (0.03% infected seeds) can result in a black rot epidemic. (Boudreux, Pollet, Whittman 1990). Seed should be tested and certified to be disease free with less than 1 in 30,000 infected seed. The organism survives in infected crop tissue left on the soil until the crop tissue rots. However, the bacteria do not survive very long in soil as unprotected free living organisms. The black rot bacteria can also infect and survive on many crucifer weeds. This also contributes to the persistence and spread of the disease. It can grow and multiply on host tissue without infecting or causing disease.

Rain splashed bacteria from contaminated plant residue left on the soil or from neighboring diseased plants is the primary method of disease spread throughout a field. (Figure1). The bacteria enter and exit through water-secreting glands called hydathodes located at the edges and tips of leaves (Figure 7).

Hydathodes often produce a drop of water during periods of high humidity early in the morning. The pathogen spreads very quickly when rain droplets contaminated with bacteria splash onto healthy leaves and enter the hydathodes. The bacteria move into the leaf veins through hydathodes and begin to multiply, rot and plug the veins. Contaminated water droplets that exude out of hydathodes of infected leaves can then be rain- splashed to other plants.

Black rot is more severe and widespread in fields that receive frequent early morning rains, particularly in May and June. Equipment, people, animals and overhead irrigation can further spread the disease. Insects can also spread the bacteria; however, their contribution to the spread of black rot is limited. Hydathodes are special glands or pores at the end of vascular tissue on leaves through which water exudes and are a natural opening for black rot bacteria to infect. (Figure 7)

Figure 7. Systematic pathogen survival pathways.



Despite all the intensive research on black rot, the disease occurs or remains latent year after year. Proper use of treatment methods calls for in-depth knowledge of the main sources of contamination (Figure 2).

- Crucifer seeds are infected by black rot below the detection level.
- Greenhouse production of transplants infected by X. campestris pv. campestris. Despite the seedlings' healthy appearance, the bacterium may often be present in a latent state on the surface of young leaves.
- After the seedlings are transplanted to the field, black rot symptoms will develop if environmental conditions are favorable.
- The presence of decomposing cabbage residues and weeds in fields can cause a secondary infection.
- The causal pathogen may be spread by rainfall, insects, agricultural machinery, humans, sprinkler irrigation; nearby cabbages may also become infected by bacteria that gain entry via hydrathodes, stomata and wounds.
- Infection may be spread to other parts of a contaminated plant by drops of water.
- Infection by secondary parasites may also occur during storage.

Disease Management in Black Rot

Black rot management begins with the identification of potential disease sources and utilising an Integrated Pest Management (IPM) strategy including host resistance, planting disease free seed, avoiding spreading the disease and proper sanitation. Sanitation is the main method that reduces, excludes or eliminates the initial sources of disease. General sanitation practices include crop rotation, disinfecting seed, rouging diseased plants, elimination of refuse piles and eradication of alternative hosts.

Seed Treatment

Seedborne inoculum significantly contributes to the spread of black rot bacteria. Growers should only plant tested certified seed < 1 infected seed in 30,000 or 0.003% contamination. (Boudreux et al., 1990). When the infection level of seed is not known or disease-free seed is not available, seed should be treated to eliminate the bacteria. Growers who purchase transplants should request proof the seedlings were grown from disease-free or treated seed. During transplanting, diseased seedlings should not be planted in the field.

Seed treatments do not always eliminate 100% of the bacteria on or in the seed, and may adversely affect seed germination and vigour. Soaking seeds in hot water at 50°C for 25–30 min. is the most effective treatment for seedborne blackrot control. Weak seed, seed stored for several years and seed of certain crucifer crops; such as, cauliflower, kohlrabi, kale, rutabaga and summer turnip, may be damaged by hot water treatment; soak for 15 min. at 50°C only.

The effect of the hot water seed treatments on every variety of each individual crucifer crop has not been investigated. Growers are encouraged to treat a small portion of seed and plant in pots to determine the effect of the seed treatment on germination and vigour, prior to treating the entire seed lot.

How to Avoid Spread of Black Rot

Use new seed trays each year to avoid contaminating this year's crop with residual black rot bacteria from the previous year. If purchasing new trays each year is not economically feasible, used trays can be sterilized with steam, boiling water or chemical disinfectants to eliminate potential contamination. Destroy infected seed trays immediately to prevent disease spread to other seedling trays. Avoid soaking crates or bundles of transplant seedlings in tubs of water before transplanting. The black rot bacteria can spread from diseased to healthy seedlings by infecting leaf scars and wounds on roots when soaked in water.

Black rot bacteria can contaminate the surface of clothing, equipment, tools and water sources. Reducing seeding rates and densities to promote good air circulation, facilitating the quick drying of plants, timing irrigation when plants will dry quickly and restricting field activities until later in the day when fields are dry will help reduce disease spread. Working in diseased fields last will also avoid disease spread from infected to non-infected fields. Wash and disinfect equipment before moving from one field to another.

Field Selection

Field selection is very important due to the distance the pathogen can spread. Whenever possible, select fields as far away from fields grown to crucifer

crops the previous year. Select fields that are well drained and will not receive run-off water from areas or fields where crucifers have been grown previously. Well drained, light soils are best for crucifer production because they can be worked early in the season and facilitate earlier planting of transplants. Planting early can help avoid disease because environmental conditions are usually not conducive for the development and spread of black rot bacteria.

Crop Rotation

Planting disease-free, treated seed or seedling transplants does not necessarily ensure a disease free crop in the field. Crop rotation is also an important management tool. Black rot bacteria can survive in infected crop tissue in soil until the crop tissue breaks down and rots. The time required for crucifer crop debris to rot varies between regions depending on the temperature, amount of soil moisture and soil type. For example, in the states of Georgia and Washington, which experience long, warm summers, it has been estimated that free-living bacteria can survive in infested soil for about 60 days, and up to 615 days in infested host debris. The bacteria can survive longer in soil during cool, wet seasons than during hot, dry seasons. In Ontario, a 3-year rotation is recommended.

Weed Control

Black rot bacteria can infect and survive on many crucifer weeds including bird rape (Brassica campestris), Indian mustard (B. juncea), black mustard (B. nigra), shepherd's purse (Capsella bursa-pastoris), globe-podded hoary cress (Cardaria pubescens), pepper grass (Lepidium densiflore) and wild

radish (Raphanus raphanistrum). Disease symptoms on weeds vary from small yellow V-shaped lesions on leaf margins to no visible symptoms. The pathogen can spread up to 30 m from infected plants (including weed hosts) to healthy plants. The pathogen not only infects and spreads from weeds to cruciferous crops, it can also survive on weed seeds and can grow and multiply on weed leaves without infecting or causing disease. Good weed control within fields will aid disease management; however, careful attention to weed control in ditches and along fencerows is also important.

Insect Control

The crucifer flea beetle (Phyllotreta cruciferae) can transmit black rot bacteria from infected plants to healthy ones; however, their importance in the spread of the disease is limited. Wounds caused by insects provide an entry point for the disease to infect plants during heavy dews or periods of rain. Insect control will help reduce the spread and severity of disease.

Cull Pile Management

Infected refuse or cull piles left in the field, provides an excellent source of the black rot bacteria. Fresh cull piles left near fields can result in severe disease epidemics during the growing season. Prepare cole crops for market away from fields, and immediately chop and bury the diseased tissue cut from plants.

Resistant Varieties

The development of crop varieties with disease resistance or tolerance to black rot has been the focus of many cole crop breeding programs worldwide. Resistance to black rot was first identified in the Japanese cabbage cultivar, Early

Fuji. Today, many crucifer hybrids with black rot tolerance are available for both fresh and processing commercial production.

Chemical Control

Soil fumigation can significantly reduce black rot bacteria. Soil fumigation is expensive and alternative methods for managing plant pathogenic bacteria are needed. For more information on chemical control options refer to OMAF Publication 363, Vegetable Production Recommendations.

Crop Nutrition

The effect of plant nutrient management on the susceptibility of host crops to black rot infection is not fully understood. A balanced nutrient program may reduce the susceptibility of plants to disease infection. Excess nitrogen promotes lush vegetative growth and may increase plant susceptibility. Micronutrients may also be involved with the disease defense mechanisms of crucifer crops.

XANTHOMONAS CAMPESTRIS

The genus Xanthomonas is a diverse and economically important group of bacterial phytopathoens, belonging to the subdivision of the Proteobacteria. Xanthomonas campestris pv. campestris (Xcc) causes black rot, which affects crucifers such as Brassica and Arabidopsis. Symptoms include marginal leaf chlorosis and darkening of vascular tissue, accompanied by extensive wilting and necrosis. Xanthomonas campestris pv. campestris is grown commercially to produce the exopolysaccharidexanthan gum, which is used as a viscosifying and stabilizing agent in many industries.

Since the early 1990s, diseases caused by Xanthomonas campestris 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. Recent achievements in the studies on resistance to black rot were reviewed. For the first time resistance genes were identified based on gene-for-gene interaction with different races of the pathogen. Some East Asian cabbage and Portuguese Penca kale cultivars seemed to carry the homologous genes for race-specific resistance. Their origin in Asian cabbages was traced to the Flat Dutch group of varieties and to heading Mediterranean kale. It is suggested that novel non-specific stem resistance found in Chinese kale, broccoli and cabbage might be an alternative means of genetic protection against the pathogen.

Incidence of black rot caused by Xanthomonas campestris pv. campestris on horticultural brassicas is well recognized worldwide. Periodical epidemics of the disease were usually ascribed to the introduction of susceptible cultivars, careless application of contaminated seeds and seedlings and weather conditions favorable for disease development. Studies on the recent outbreaks caused by X. campestris on oilseeds suggested that spreading of new highly aggressive variants of the pathogen was the main reason for these epidemics. However, breeding of cabbage for resistance to black rot has been undertaken without recognition of the existence of pathogenic variants (races). As a result, control of the disease by the introduction of some resistant cultivars may not be effective.

Xanthomonas campestris pv. campestris (Xcc) belongs to the genus that causes diseases on at least 124 monocotyledonous and 268 dicotyledonous plant species including all major crop plants. According to Lambe, (1992) recent reclassification based on DNA analysis, Xcc was assigned to the same genetic group as that of other pathovars infecting a wide range of crucifers as systemic or leaf pathogens. The clear difference between leaf spot and black rot symptoms was attributed to the expression of a few genes, present in these pathovars. Factors responsible for the pathogenicity of Xcc include plant-stimulated proteins produced by pathogenicity genes targeted to plant nucleus, several enzymes and extra-cellular polysaccharides.

Most *Xanthomonas* bacteria produce yellow, membrane-bound, brominated aryl-polyene pigments referred to as xanthomonadins. Xanthomonadins are unique to *Xanthomonas* bacteria and serve as useful

chemotaxonomic and diagnostic markers. With methods of artificial infection, xanthomonadin-deficient strains were not affected in pathogenicity, symptomatology, or in plant growth. Thus, the xanthomonadins apparently are not important to the pathogen after infection of the host plant (Lambe et al., 1992). *Xanthomonas campestris* pv. campestris, the causal agent of black rot of crucifers and one of the most serious disease problems in crucifer production, naturally infects its host via hydathodes or wounds in the leaves.

Since the pathogen can remain in soil even in plant debris only for 1 or 2 growing seasons, survival in contaminated seeds and on weed crucifers is considered to be most essential for the cycle of the disease. In Southeast Asia, although pak-choi, pet-tsai and other oriental brassicas are less damaged by black rot than vegetables of the B. oleracea group, they could become a source of inoculum.

When the U.S. military became involved in the Korean war, scientists at NCAUR quickly made an all-out effort to develop an economical way to mass-produce dextran, a polysaccharide. Dextran is produced when a strain of the bacterium Leuconostoc mesenteroides acts on cane or beet sugars. Dextran was quickly approved as a blood extender for use in military medicine in 1950 and for civilian use in 1953.

Xanthan Gum

A common thread in the penicillin and dextran developments was microbiological expertise and resources. Shortly after the Korean war, the dextran research team tapped a strain of the bacterium Xanthomonas campestris from

among microbes at NCAUR to develop xanthan gum, a thickening substance with food and industrial applications.

Xanthan gum is produced, during a natural process, from the fermentation of a sugar, in the presence of Xanthomonas campestris bacteria. Industrially, the carbon-based source is sweet-corn. After filtration and sterilization, xanthan is collected in the fermentation juice by alcoholic floculation. Then the coagulum is separated, washed and water is squeezed out ; then it is dried and pulverised. Product is a pale beige powder.

The history of xanthan gum is recent. At the end of the 50s, the research laboratories of the U.S. Department of Agriculture were working on microbial biopolymers industrial applications. They discover polysaccharide produced by Xanthomonas campestris bacteria found on the cabbage. This gum has remarkable properties in an aqueous medium especially for emulsions and suspensions stabilization. In 1969, xanthan gum is approved by the US Food and Drug Administration (FDA) as a food additive. (Lambe et al., 1992)

Xanthan gum applications concern a great number of sectors such as food industry, cosmetics, hygiene and care products. Various industrial applications and agri-business applications are by far the most numerous.

CHAPTER 3

DESIGN OF THE STUDY

The decrease in stratospheric ozone has prompted renewed efforts in assessing the potential damage to plant and animal life due to enhanced levels of solar Ultraviolet-B (UV-B, 280-320 nm) radiation (Caldwell, 1999). The effect of UV-B enhancements on plants includes reduction in yield and quality, alteration in species competition, decrease in photosynthetic activity, susceptibility to disease, and changes in plant structure and pigmentation (Madronich, McKenzie, Bjorn, and Caldwell, (1998). Some species show sensitivity to present levels of UV-B radiation while others are apparently unaffected by rather massive UV enhancements (Becwar, Moore, Bureke, 1999). This issue is complicated further by reports of equally large response differences among cultivars of a species (Biggs et al., 1989; Teramura and Murali 1986). About two-thirds of some 300 species and cultivars tested appear to be susceptible to damage from increased UV-B radiation. Crops such as soybean, winter wheat, cotton, and corn are susceptible to damage from increased UV-B radiation.

All effects of elevated UV-B on plants should be considered in the context of other factors such as water stress, increased atmospheric CO2, air pollution, and temperature. The effects of UV-B on plants have been studied mostly under greenhouse, while a few experiments conducted under field conditions (Krupa, Kickett, 1989). There are also few studies that have examined the joint effects of UV-B on plant diseases. The effect of UV-B on plant growth and productivity

varies seasonally and is affected by microclimate and soil fertility. For instance, soybeans are less susceptible to UV-B radiation under water stress or mineral deficiency, but sensitivity increases under low levels of visible radiation (Teramura, 1983). Continued studies over many growing seasons are crucial in any UV-B impact assessment of agricultural productivity.

Materials and Methods

The following section contains only discussion of materials and methods used to determine the effects of UV-B on Black Rot. Twenty-four cabbage plants which were obtained from a farm in Wharton, Texas were transplanted to twelve-inch pots using a 1: 5 mixture of soil infected with Xanthomonas campestris (Xc) and methane mix 300 potting soil. The planted cabbage was then moved to an unshaded greenhouse for duration of the experiment. The twenty-four cabbages were then separated in three experimental groups of eight. Each experimental group contained one control plant. The natural photoperiod ranged from 10 to 12 hours daily. Day temperatures ranged from 75 to 98 F and night temperatures ranged from 70 to 80 F. Each group was watered as needed and fertilized once a week with a 20:20:20 (nitrogen: phosphorus: potassium) commercial fertilizer. Plants were irradiated daily with Ultraviolet-B after the appearance of Black rot. The plants were randomized to eliminate position effects in the greenhouse. Six filtered Westinghouse FS-40 sunlamps artificially supplied the UV-B irradiation. Each plant group received between (280-320 nm) of UV-B radiation for a fourteen-day period for exactly 4 hours daily. The total weekly-elevated radiation treatment time was 28 hours and the total experiment treatment time was 56

UV-B on plant diseases. The effect of UV-B on plant growth and productivity varies seasonally and is affected by microclimate and soil fertility. For instance, soybeans are less susceptible to UV-B radiation under water stress or mineral deficiency, but sensitivity increases under low levels of visible radiation (Teramura, 1983). Continued studies over many growing seasons are crucial in any UV-B impact assessment of agricultural productivity.

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treatment time was 28 hours and the total experiment treatment time was 56 hours. The entire experiment took place in a local greenhouse under controlled conditions.

The control plants in each group were treated with only natural light after the appearance of black rot. For the control, radiation from natural light was filtered with a presolarized 0.13-mm Mylar Type S, which absorbs almost all radiation below 320 nm. For UV-B radiation treatment, artificial light was filtered with presolarized 0.08-nm cellulose diacetate, which transmits wavelengths down to 290 nm. The spectral irradiance of the lamps was determined by using an Optronics Model 742 Spectroradiometer (Optronics Laboratories, Inc., Orlando, FL). Radiance filtered through cellulose diacetate supplied a total daily weighted irradiance of 11.6 kJ•m

The intensity of UV-B may vary by changing the height between the lamp source and the plant canopy. Because different biological processes exhibit different degrees of sensitivity to different wavelengths of UV-B, a mathematical response function, the action spectrum, was used as a weighting factor to adjust the measured UV-B flocculation. The various sources of uncertainties in calculating biologically effective UV-B flocculation should be considered.

CHAPTER 4

RESULTS AND DISCUSSION

The elevated UV-B radiation generally has negative impacts on plant growth, yield and quality of cabbage plants. However, in all of the groups infected with Xanthomonas campestris, bacteria growth was reduced relative to the control. With enhanced UV-B radiation, photosynthesis decreases, plant height and leaf area decreased, dry matter production, yield and quality were reduced in many crops. Thus, the overall quality of each cabbage plant does not meet most standards. These responses vary with different plant species. Some are very sensitive and some are less sensitive. In the study conducted by Tevini et al. (1991) plant height, leaf area, and the dry weight of sunflower, corn, and rye seedlings were significantly reduced with enhanced UV-B radiation.

The results that follow indicate a change in bacteria growth area. The bacteria growth area was determined with the slide rule method. The measurement units were taken in cubic centimeters. From the bacteria growth rate, it was possible to determine the change growth, which is described as the *Bacteria Growth Reduction (BGR).* The BGR is the numerical difference between the control of the respective group and the Bacteria Growth Area of a given plant for that group

Table 1. Group One –Bacteria Growth Results

Cabbage Plant Number	Bacteria Growth Area (in centimeters)	Bacteria Growth Reduction (in centimeters)
1	19.7 cm	1.8 cm
2	18.2 cm	3.3 cm
3	17.6 cm	3.9 cm
4	17.1 cm	4.4 cm
5	19.1 cm	2.4 cm
6	18.5 cm	3.0 cm
7	18.8 cm	2.7 cm
8	18.1 cm	3.4 cm

Bacteria Area for Experimental Control = 21.5cm

Average Bacteria Growth Area = 18.3 cm

Average Bacteria Growth Reduction = 3.11 cm

*** Bacteria Reduction Percentage = 14.8%**

***Bacteria Reduction Percentage Calculated by**

$$\frac{\text{Bacteria Area Control - Average Area Bacteria}}{\text{Bacteria Area Control}} \times 100 = 14.8\%$$

Figure 8. Graph One - Bacteria Growth Results of Group One

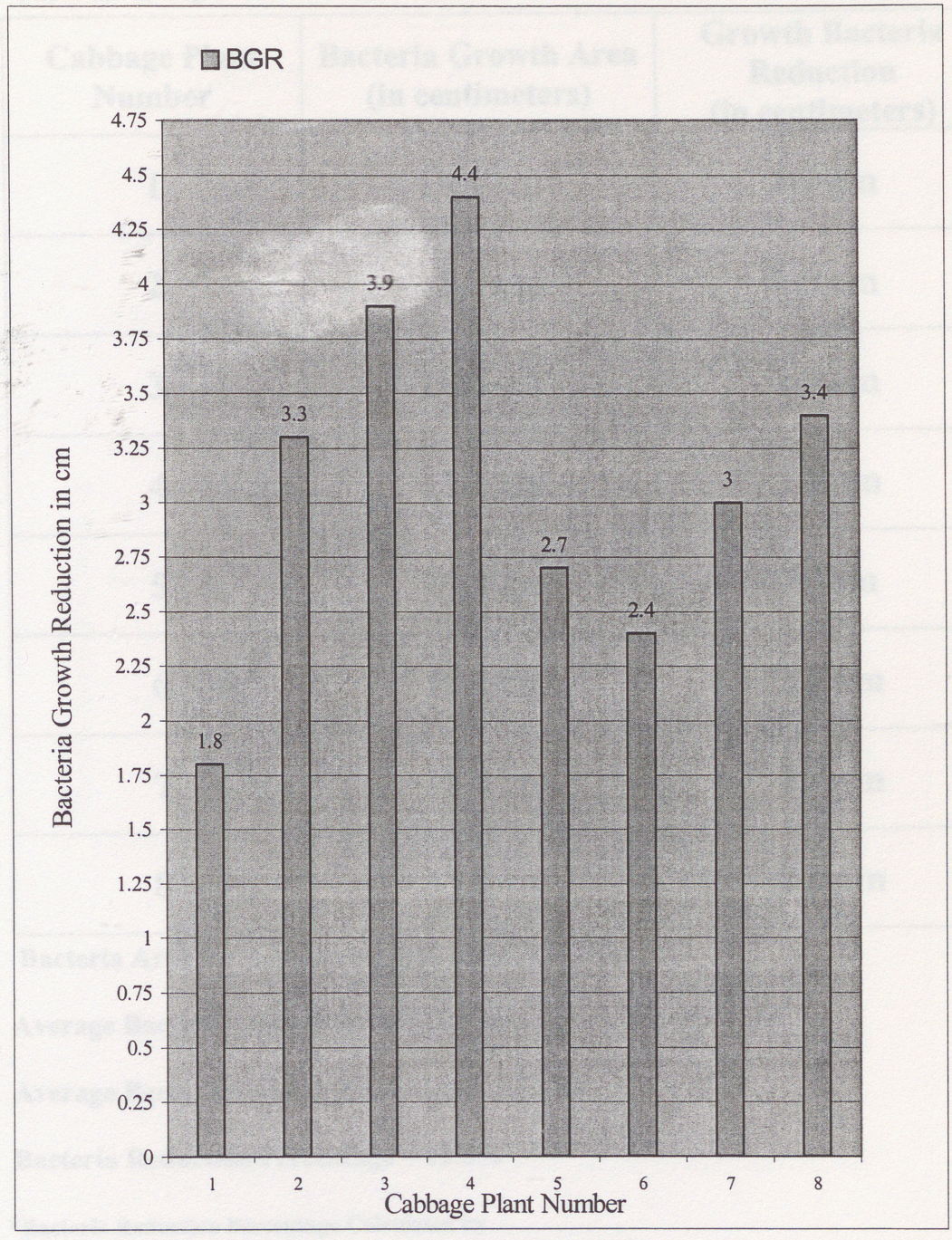


Table 2. Group Two – Bacteria Growth Results

Cabbage Plant Number	Bacteria Growth Area (in centimeters)	Growth Bacteria Reduction (in centimeters)
1	19.1 cm	1.7 cm
2	18.2 cm	2.6 cm
3	18.6 cm	2.2 cm
4	17.1 cm	3.7 cm
5	19.1 cm	1.7cm
6	18.1 cm	2.7 cm
7	18.0 cm	2.8 cm
8	17.9 cm	2.9 cm

Bacteria Area for Experimental Control = 20.8cm

Average Bacteria Growth Area= 18.2 cm

Average Bacteria Growth Reduction = 2.53 cm

Bacteria Reduction Percentage = 12.5%

***Bacteria Reduction Percentage Calculated by**

$$\frac{\text{Bacteria Area Control - Average Area Bacteria}}{\text{Bacteria Area Control}} \times 100 = 12.5\%$$

Figure 9. Graph Two -Bacteria Growth Results of Group Two

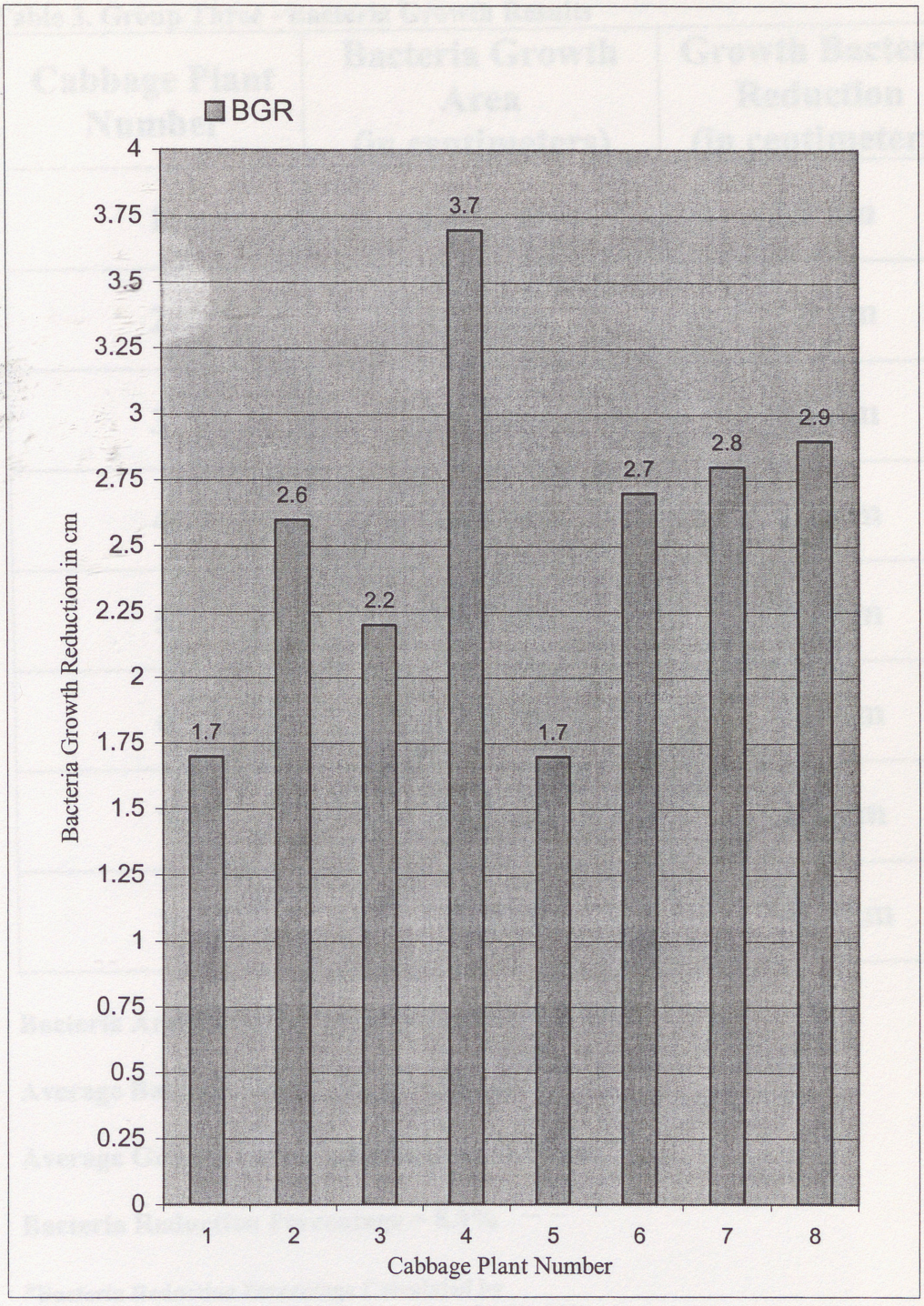


Table 3. Group Three - Bacteria Growth Results

Cabbage Plant Number	Bacteria Growth Area (in centimeters)	Growth Bacteria Reduction (in centimeters)
1	18.1 cm	3.1 cm
2	19.2 cm	2.0 cm
3	18.7 cm	2.5 cm
4	20.1 cm	1.1 cm
5	19.1 cm	2.1 cm
6	18.3 cm	2.9 cm
7	19.1 cm	2.1 cm
8	21.9 cm	-0.7 cm

Bacteria Area for Experimental Control = 21.2cm

Average Bacteria Growth Area = 19.3 cm

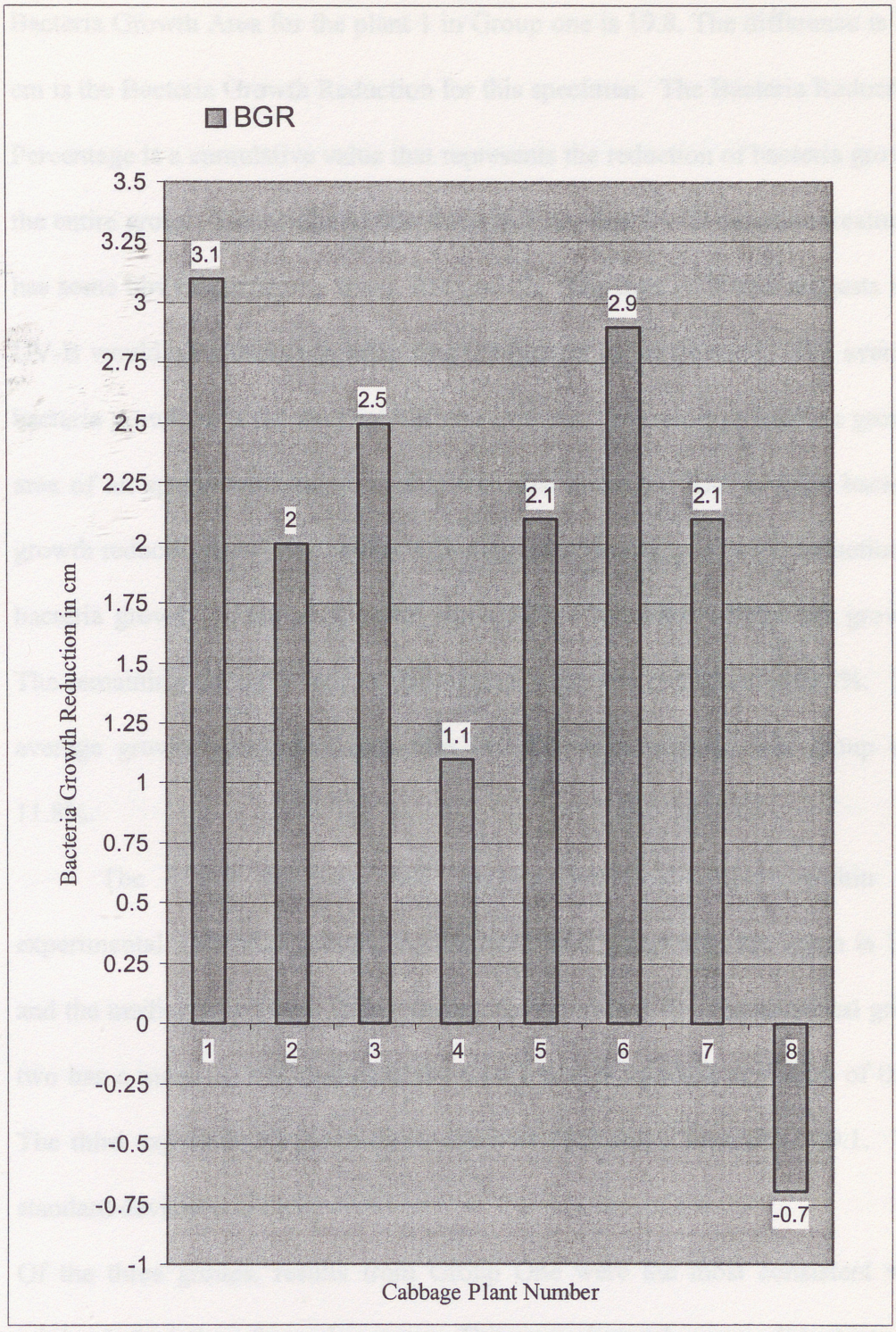
Average Growth Bacteria Reduction = 1.88 cm

Bacteria Reduction Percentage = 8.9%

***Bacteria Reduction Percentage Calculated by**

$$\frac{\text{Bacteria Area Control - Average Area Bacteria}}{\text{Bacteria Area Control}} \times 100$$

Figure 10. Graph Three -Bacteria Growth Results of Group Three



For example the control value for group one is 21.5 cm and the Bacteria Growth Area for the plant 1 in Group one is 19.8. The difference is 1.8 cm is the Bacteria Growth Reduction for this specimen. The Bacteria Reduction Percentage is a cumulative value that represents the reduction of bacteria growth the entire group. The results of this study indicate that UV-B radiation treatment has some obvious effect on Black Rot growth. The data collected suggests that UV-B would be effective in inhibiting the growth of the bacteria. The average bacteria growth area for the controls was 21.6 cm. The average bacteria growth area of all specimens treated with UV-B was 18.6 cm. The average bacteria growth reduction rate was 2.6 cm. In Group 1, there was a 14.8% reduction in bacteria growth. In Group 2, there was a 12.5% reduction of bacteria growth. The remaining Group 3 had the least bacteria growth reduction with 8%. The average growth percentage reduction for the entire experimental group was 11.8%.

The UV-B radiation had varying statistical effects within the experimental individual groups. For experimental group one, the mean is 18.3 and the median was 18.35. The standard deviation is 0.78. Experimental group two has a mean of 18.2 and median of 18.1 with a standard deviation of 0.63. The third experimental group has a mean of 19.3 and a median of 19.1. The standard deviation is 1.13.

Of the three groups, results from Group One were the most consistent with minimal deviation from the mean. This experimental group did show the greatest reduction of bacteria growth. Group two did show a major variation with plants one and five. Both had a similar 19.1 cm. This trend of similar

values only occurred in this group. There is no explanation for this phenomenon however; it qualifies the randomness of the results. The plants in Group two were the overall healthiest at end of the 14-day experiment period. In addition the Black Rot bacteria had only infected small portions of the root. In the two other Groups roots were thoroughly infected. This would signify a greater infection in these groups.

In Group three, there is a much different bacteria growth trend. In this group, specimen eight had a Bacteria Growth Area of 21.9 cm. The control for Group three had a Bacteria Growth Area of 21.2 cm. Based on these values, specimen eight had a negative growth reduction, indicating in this particular trial that the bacteria specimen outgrew the control for that group by 0.7 cm. It is quite possible to attribute this trend to a number of possibilities. The obvious would be an experimental error; however, it is probable that extreme variation in BGR is due to the condition of this plant specimen. In spite of the plant specimens being harvested and treated in the same method, each plant does not develop the same. It is possible that this plant lack, a well-defined cuticle, which would minimize the disease growth. The cuticle acts as a barrier to UV-B Pierce, (1987) found that greenhouse-grown plants are known to have a much thinner and less well-developed cuticle than field-grown plants and thus might exhibit greater sensitivity to bacteria and disease. In terms of experimental error being responsible for this phenomenon, it is possible that the Xanthomonas campestris was administered in a higher concentration in this plant specimen.

The results obtained in this investigation would vary dramatically when comparing the effects of UV-B exposures in a greenhouse to those under field

conditions. Such differences may well arise not only from differences in the microclimate but, in varying heat energy as well.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

There has been no network for monitoring UV-B radiation on plants and crops. Long-term UV-B data are sparse and not very reliable. Nevertheless, numerous investigators have examined the effects of UV-B radiation on crops in artificial exposures, but uncertainties in the relevance to climate change of much of the information obtained remain. According Krupa et al., (1989), the transfer of results from growth chamber or greenhouse experiments to the natural environment has been particularly difficult. This appears to be due to the differences in the characteristics of plants grown under these environments. Studies of the effects of UV-B on physiological processes such as photosynthesis are appropriately examined under controlled environment conditions. However, the integration of their effects on the plants infected with various diseases should ultimately lead to more investigations using crops growing under controlled conditions. Knowledge of the effects of UV-B on plant diseases also has uncertainties related largely to the lack of information about plant responses under various field conditions. Here the problem is not one of interrelating growth and field observations, but concerns the relevance of the results from the most frequently used open-top exposure chamber method. There is no question of the effects of elevated UV-B on plant disease. The results obtained in this study support this idea and therefore, the fact remains

that the results can be validated and show considerable significance.

Perhaps the most pressing need at the moment is to obtain field information about the effects of UV-B on disease in cabbage in one or more of the different scenarios. Such future studies should include elevated levels of carbon dioxide and oxygen. While such information is needed for direct effects on cabbage crop species, the studies must also include information about the possible long-term effects on growth, joint effects with other pollutants, incidence of pathogens and insect pests and intra-species competition. These studies should also permit the gathering of information about the processes involved, such as the induction of morphological changes. In contrast to the mechanisms affecting growth and development, ongoing studies at the biochemical and metabolic level are needed in order to provide a sound understanding of the disease black rot. In view of the evidence of this study that suggests that UV-B has some inhibitory effects on Black Rot growth in greenhouse conditions, it appears that as a treatment, UV-B irradiation has some usefulness in bacteria management.

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