KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

SURVEY AND ESTIMATION OF PATHOLOGICAL AND EDAPHIC CAUSES OF CITRUS PRE-HARVEST FRUIT DROP IN THREE MAJOR CITRUS GROWING AREAS IN ASHANTI REGION OF GHANA.

BY

DANIEL ADU BOAKYE

A Thesis Submitted to the Department of Horticulture, Faculty of Agriculture, College of Agriculture and Natural Resources in Partial Fulfillment of the Requirements for the Award of the Degree

MASTER OF PHILOSOPHY (FRUIT SCIENCE)

OCTOBER, 2016

DEDICATION

This work is whole heartedly dedicated to God Almighty, my uncle, Mr. Kwaku OwusuKarikari and my mother, Mrs. Beatrice Asiamah**.**

DECLARATION

I, Daniel Adu Boakye, declare that this submission is my own work towards the M.Phil. (Fruit Science) and that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

STUDENT: DANIEL ADU BOAKYE

Sign: ______________________________

Date: ______________________________

SUPERVISOR: PROF. PAUL YAW ADJEI

Sign: ______________________________

Date: ______________________________

CO- SUPERVISOR: DR. CHARLES KWOSEH

Sign: ______________________________

Date: ______________________________

DR. B. K. MALEEKU (HEAD)

DEPARTMENT OF HORTICULTURE

Sign ______________________________

Date: ______________________________

ACKNOWLEDGEMENT

Nyansa buakwa Nyame, thank you for your amazing grace to me throughout my studies. It is by you, of you and with you that I have come this far. Jehovah Elohim, all my worship and praise are unto you alone. Indeed, great is thy faithfulness.

My appreciation goes to Mr. Paul Yaw Adjei and Dr. Charles Kwoseh for their immense contribution and supervision of my research work. I am really grateful, Mr. Adjei, for your financial support towards this research and may God extremely bless you.

Special gratitude goes to my uncle, Mr. Kwaku Owusu-Karikari and my mother, Mrs. Beatrice Asiamah (Mama), my grandmother, Maame Abena Konadu and my sister, Harriette Owusu-Karikari and the entire family. I do recognize your contribution to my development so much.

In addition, I thank all the following people who have contributed in diverse ways towards my research; Dr. E. Osekere, Mr. Thomas Adjei-Gyapong (Crop and Soil Sciences, KNUST), Mr. Emmanuel Ata Aidoo, Maxwell, Mad. Zipporah, all of Crop Research Institute and Mr. Peter Ofori, Stella Adjeiwaa-Sefa, Isaac Owusu Ansah, Mr. Charles Asante and Mr. Anthony Abutiate from Soil Research Institute, Kwadaso. I do appreciate your technical support so well. Again, much appreciation goes to Adwoa Sarfoah and Mr. Bismark Owusu Sekyere of Pathology lab, Faculty of Agriculture, KNUST.

To my friends, Kofi Asare Mensah, Akua Darkowaa Poku, Solomon Barmouny, Tony Yeboah, Mark Mensah and Julius Zilevu, I bless God for meeting you in my life.

Lastly, all the staff of Department of Horticulture, KNUST and farmers who assisted me in diverse ways may God bless you all.

TABLE OF CONTENTS

ABSTRACT

Citrus pre-harvest fruit drop is a major problem to citrus farmers in the Ashanti Region of Ghana. Farmers' perception was assessed, pathological and soil-plant nutritional causes were researched into in three major citrus growing areas in Ashanti Region (Bekwai Municipal, Ahafo Ano South and Adansi North). Sixty farmers were interviewed with structured questionnaire to ascertain farmers' views on the causes of citrus pre-harvest fruit drop and its management practices. Severity of the pre-harvest fruit drop was assessed by counting from all the selected areas for two varieties (late valencia and red blood). The estimation was done from mid-August to mid-September, 2015 for the red blood variety and mid-December, 2015 to mid-January, 2016 for late valencia. Using grid method, matured leaves (30 per variety from a farm) from non-bearing terminals and 12 soil cores composited for representative samples were taken from an estimated one hectare field.

Twenty-four fruit samples each (dropped and un-dropped) were taken from twelve trees for analysis for each of the varieties. Plant and soil samples were analysed for N, P, K and Zn respectively at CSIR-Soil Research Institute, Kwadaso. Organic Carbon concentration was also determined. Lesioned fruits were sent to Plant Pathology laboratory of the Faculty of Agriculture, KNUST for isolation and identification of pathogen(s) associated with citrus pre-harvest fruit drop. Data obtained from questionnaire and nutritional analyses were subjected to SPSS and Genstat statistical analysis respectively. Ninety five percent of farmers were of the opinion that citrus black spot disease causes citrus pre-harvest fruit drop. Thirty-three percent of farmers indicated no control measure for fruit flies while 28.3 % of farmers combine bait application and pheromone trap in their orchards. The black spot symptoms were perceptibly seen on matured green fruits then forces the fruits to ripe, which is called '*kyembedie*' in the local language. However, 55% indicated that no control measure has been undertaken against the disease. Red blood variety (228) dropped more than late valencia (109) in all the major citrus growing areas. From the leaf analysis, the nutritional status for N and P at Ahafo Ano South had the highest concentrations (2.98 % and 0.17 %) whilst Bekwai Municipal had the lowest concentrations (2.29 % and 0.14 %) respectively. For K; Ahafo Ano South had the highest concentration (0.60 %) and Adansi North had the lowest (0.42 %). With zinc, Adansi North had the highest concentration (6.7 mg/kg) and Ahafo Ano South had the lowest concentration (4.9 mg/kg). It was observed that the direct relationship (r) between leaf and dropped fruit peels for zinc (0.87) and nitrogen (0.20) respectively resulted to the higher mean fruit drop (108) for late valencia. Also, the moderate the direct association for zinc (0.63) and the inverse the relationship for nitrogen (-0.37) resulted to higher fruit drop (228) at Ahafo Ano South for red blood variety. The pathogens that were

found on the dropped fruits with black lesions are *Phyllosticta citricarpa*, *Aspergillus niger*, *Aspergillus flavus* and *Collectotrichum gloeosporoides* from both late valencia and red blood varieties from all the selected areas. The highest level of incidence of citrus black spot lesions for late valencia (89.64 %) was at Adansi North and red blood variety (92.18 %) at Bekwai Municipal.

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Citrus, from the family Rutaceae and subfamily Aurantioideae is made up of three types: *Citrus*, *Fortunella (Kumquat)* and *Poncirus*. There are three genera and eighteen defined species, but other natural mutations exist resulting in numerous hybrids which are widely spread throughout the world (Guo and Deng, 2001). Citrus is widely grown in tropical (including Ghana), and subtropical regions (Piccinelli *et al.*, 2008). There are several varieties of citrus and one of the most commonly grown variety is sweet orange (*Citrus sinensis*). The origin of sweet orange is not known, though it is believed to be obtained from interspecific hybridization of some primeval species (Xu *et al.*, 2013).

Citrus are evergreen plants that give fruits of different forms and sizes (round to oblong). The fruits are fragrant and have good flavour and juice. It grows particularly well in areas with sufficient rainfall and/or irrigation to sustain growth and where freezing is not severe enough to kill the trees (Whiteside *et al*., 1988).

Citrus has high nutritional importance due to the presence of functional food ingredients and antioxidant, neutraceuticals or phytochemicals. Citrus fruits are the main source of phytochemical nutrients and have value for their nutritious and antioxidant properties (Keys, 1995). In addition, it is appreciated that biologically active and non-nutrient compounds such as insoluble dietary and soluble fibres are known to be helpful in reducing the risk for cancers, arthritis, obesity and coronary heart diseases (Crowell, 1999). Citrus fruits are a good source of dietry fibre, carbohydrates, minerals and vitamins. Economically, *Citrus sinensis* is an important fruit crop, where around 194.39 million metric tonnes was produced worldwide (USDA FAS, 2014). Brazil and the United States of America supply half of the total (Goudeau *et al.*, 2008; Bernardi *et al.*, 2010). Xu *et al.*

(2013) reported that among different citrus production sweet oranges ranks first among tree fruit crops. In 2013, FAO reported the continental total production of sweet orange and they are America (47.7 %), Asia (31.4 %), Africa (11.7 %), Europe (8.6 %) and Oceania (0.6 %) but the leading countries supplying between 8-20 million tonnes are Brazil, USA, China and India. Ghana is one of the countries with the highest yield ranging from 300 – 400 thousand tonnes in addition to South Africa, Albania, Turkey and Indonesia (FAOSTAT, 2013).

1.1 Problem Statement/Justification

Despite production volume, numerous economical and health benefits of sweet orange challenges affect the expected yield worldwide. Almost every country in the tropics, subtropics or temperate regions that cultivate citrus (sweet orange) has a perculiar problem. The problems vary from damage by diseases or pests attack, cost of labour and production, low yields, climate change and pre- and post-harvest losses leading to severe economic losses.

Though, natural physiological drop of immature fruits is known to be normal as part of the plant survival mechanism however massive pre-harvest (mature) fruit drop has been a recognized problem in fruit production such as apple, citrus etc. for many years and has been discussed in early horticultural literature review (Ward, 2004). There are many fruit abscission waves in addition to those that organize balance between vegetative and flowering density. There are frequent problems with mature citrus fruits that drop before harvest. Matured fruits abscission represents a huge loss to sweet orange farmers since they are not edible and have poor taste quality with low economic value. Pre-harvest fruit drop compels farmers to harvest earlier than desired to ensure that an optimum number and quality of fruits (Karim and Neven, 2012) for consumers and industries are obtained.

Pre-harvest fruit drop has led to loss of produce and income among farmers and low revenue to various economies or states such as Florida, California, Thailand, Brazil and many other tropical countries (of which Ghana is of no exception) and sub-tropical regions of the world. In the last few years, sweet orange farmers in Ghana especially those in Central, Ashanti and other citrus growing regions are being challenged with citrus pre-harvest fruit drop. Some farmers are abandoning their farms, cutting down citrus trees to cultivate other crops such as cocoa. Though, it is generally suspected that pre-harvest (mature) citrus fruit drop is caused by imbalances in soil-plant nutrition and plant hormone as well as insect pest infestation and disease infections (Davies, 2002). Nartvaranant, (2012), reported that citrus greening disease that is known to cause preharvest fruit drop in Thailand is caused by low total non-structural carbohydrate concentrations, low plant nutrients and low IAA concentrations which contribute to preharvest fruit drop in the pummelo cultivars. Ashraf *et al.* (2012), in Pakistan conducted a study on causes of yield losses of citrus (kinnow) and attributed this mainly to inadequate nutrient management in the citrus farms. EMQAP-MOFA, (2013) in Ghana report indicated that insects; *Bactrocera invadens* and *Ceratitis capitata* caused substantial loss in fruits and vegetables cultivated in Ghana. Mintah *et al*. (2012), reported that inadequate soil water prior to fruit maturity result in poor holding of fruit and result in high fruit drop. Again, citrus pre-harvest fruit drop that increases crop losses is linked with citrus black spot and have been estimated at 71% in Ghana (Brentu *et al*., 2012) and as much as 80 % fruit loss in untreated groves in Australia and South America (Calavan,1960). Despite policy makers' attention on agricultural diversification and commercialization of some horticultural crops such as citrus and pineapple, there are major challenges confronting farmers in their productions and examples are pest and disease attack and pre-harvest fruit drop. Therefore, this study seeks to survey on farmers' perception and their management practices, the severity of fruit drop and the pathogens associated and nutritional status on citrus (*Citrus sinensis)* farms in Ahafo Ano South, Bekwai Municipal and Adansi North districts in the Ashanti region of Ghana.

The objectives of this research were to:

- assess farmers' perception on the causes of citrus pre-harvest drop and their management practices
- determine the severity of citrus pre-harvest fruit drop in the major citrus growing areas
- determine the soil and plant nutritional status of nutrients associated with citrus pre-harvest fruit drop.
- identify pathogens associated with citrus pre-harvest fruit drop.

CHAPTHER TWO

LITERATURE REVIEW

2.1 Botany, Origin and Uses of Sweet Orange

South East Asia is the origin of most citrus species where it has been cultivated for many years, but currently grown worldwide in tropical, sub-tropical and some warm temperate regions commercially (Ehler, 2011). Much as the origin of most citrus cultivars is unknown but the ancient citrus relatives are native to South East Asia, New Caledonia, China, the Malay Archipelago and Australia (Atta *et al.*, 2012).

There are four major categories of citrus cultivars according to origin (Mediterranean and Spanish oranges), fruit morphological characteristics, chemical constituents, for convenience and taste (Ehsani, 2007). The round or valencia oranges are important and key portion of sweet orange hectarage grown commercially worldwide. The navel oranges named because of its shape (segmented skin looks like a human navel) originated as a single mutation in a Brazilian monastery in 1820. Blood oranges were named after their red flesh fruit that range from sweet to tart. There are three kinds of blood oranges and they are moro, tarocco and sanguinello with each differing in size, origin and taste. Acidless oranges are planted primarily for backyard use and are not of commercial importance (Davies and Albrigo, 2006; Ehsani, 2007).

Citrus is evergreen tree with height between 7.5 m - 15 m. Orange produces leathery, dotted with translucent oil cells and green leaves of different shapes. The fruit is composed of two distinct regions anatomically, the pericarp also known as the peel or rind, and the endocarp, called pulp and juice sacs. The pericarp consists of the outer flavedo or epicarp made of parenchyma cells and cuticle (Goudeau *et al.*, 2008). The mesocarp that is under the flavedo is composed of tubular cells combined together to create the tissue mass compressed into the intercellular area. The pulp of the fruit is juicy and sweet and mostly divided into 10 to 14 segments. The ripe fruit is a hesperidium which is a berry type with multiple seeds and is fleshy. Fleshy juice sacs accumulate organic acids, sugars and large amount of water causing difficulties in the extraction of proteins and nucleic acids. Sweet oranges are consumed as fresh fruit or juice; whiles other citrus products include pasteurized juice, pulp for cattle feed, pectin, essential oils and flavonoids from peels (Samson, 1986).

2.2 Climate Conditions and Soil Requirement of Citrus

Citrus species thrive in a wide variation of soils from sandy soils to those with high level of clay. However, well-drained, medium-textured, deep and fertile are good for growing citrus within a pH range of 5.5 to 6.0. Waterlogged or saline soils are not suitable. Citrus roots do not grow well in acidic soil and might lead to copper toxicity. When pH is above 6, there is trace elements fixation (such as zinc and iron) which causes trees to develop deficiency symptoms (www.infonet-biovision.org). Climatic conditions that favour citrus optimal growth 2° to 30° C and an altitude of 2100 m above sea. However, individual species and varieties decrease in susceptibility to low temperatures in the following sequence: grapefruit, sweet orange, mandarin, lemon/lime and trifoliate orange as most hardy. Blood orange varieties are limited to mediterranean-type climates whiles late valencia variety prefers tropical climate (Morton, 1987). Temperature plays essential role in the production of high quality fruits. Typical colouring of fruit takes place if night temperatures are favourable coupled with low humidity during ripening time. Exposure to strong winds and temperatures above 38° C might cause fruit drop, scarring and scorching of fruits. In the equatorial belt, the tropical climate zone experiences humid and hot weather when there is abundant rainfall due to the active vertical uplift or convection of air that occurs. In addition, when there is considerable sunshine with excessive rainfall it gives an ideal growing condition for profuse vegetative growth. The maximum temperature of 32°C is more common during daytime and minimum temperatures fall not lower than about 22°C. This temperature level is maintained with little or no variation throughout the year (http://www.ecoca.ro/meteo/tutorial/Climate/Older/Climate_Zones.html). In the tropics, the high lands provide the favourable night weather for orange colour and flavour.

Mediterranean-climate regions are found between 31^o and 40^o latitude north and south of the equator. The mediterranean-climate temperature range mostly falls within -1^o and 38^{o}C (californiarangeland.ucdavis.edu/Mediterranean-climate).

2.3 Health and Nutritional Benefits of Citrus

It is established that citrus is a rich source of vitamins, dietary fibre and minerals that are necessary for growth, development as well as nutritional well-being (FAO, 1998). Citrus fruits contain elements such as antioxidants, vitamin C, carotenes, folate, potassium, fibre and many non-nutrient phytochemicals. Citrus fruits have the highest antioxidant activity of all fruit classes. Antioxidant is very essential to various enzyme systems by boosting the immune system that protects against cancer, heart disease, cataracts, and degeneration of the macular area of eyes and infection. It also helps in the absorption of iron and zinc in other foods. Carotenoids such as beta-carotene serve as precursor for vitamin A. Citrus fruit has high level of folic acid which prevents neural tube defects in children, stabilizes genetic material and protective against cancer. A mineral in citrus is responsible for the fluids regulation in the body. High potassium and low sodium level may help in prevention of high blood pressure (Baghurst, 2003). Essentially, dietary fibre decreases food transit time in the gut, improves gut microflora, helps lower blood fats and relieve gastric conditions such as constipation.

Moreover, non-nutrient compounds such as phytochemical antioxidants, soluble and insoluble dietary fibres are helpful in reducing the risk for cancers and many chronic diseases (Crowell, 1999). Citrus flavonoids prevent cancer through selective cytotoxicity, anti-proliferative actions and apoptosis (Elangovan *et al.*, 1994). Flavonoids can protect the DNA by interacting directly with the tumor agents in the induced chromosomal aberrations by bleomycin (Heo *et al.*, 1994). Citrus species are rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, pectin, beta-carotene and amino acids and fibre. A single fruit has about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, blood clot inhibiting and antioxidant properties (Cha *et al*., 2001). Webber *et al.* (2006) reported that citrus fruits are important for the prevention of scurvy which results from lack of vitamin C in diets.

2.4 Fruit Growth and Abscission

Fruit formation is a genetic developmental program exhibited over a period of time. Flowering takes place in spring and the subsequent fruit formation that extends till midwinter under subtropical weather conditions. In tropical climates, bud sprouting and flowering occur without interruption throughout the year although the main bloom comes about during the spring (Spiegel-Roy and Goldschmidt, 1996). In tropical conditions, citrus flowers in response to drought periods. In addition, low temperature and water deficit are being recognized for a long time as another strong flowering inductor in citrus.

Water deficit increases the ratio of floral shoots and the total number of flowers (Southwick and Davenport, 1986). However, full ripening or maturity in early varieties are reached in September while in late species it can be prolonged until the onset of dry period and example is late valencia. Citrus fruit growth and development occurs after a typical sigmoid growth curve that is divided into three clear-cut stages (Iglesias, *et al*., 2007).

The growth at the initial phase is about two-month interval of cell division and slow growth between anthesis and June drop period. Thereafter, there is rapid growth period (phase II) that leads to water accumulation and cell enlargement during four to six months where the fruit increase in size. At ripening period, growth (phase III) is often arrested and fruits undergo a non-climacteric process of ripening. The reproductive organs abscission is continuous during phase I where two waves of elevated abscission take place at the onset of phase I and during the transition to phase II (Iglesias *et al.*, 2007). Several factors (endogenous and exogenous) configure the decision of aborting growth. The period of fruit set ending coincides with the metabolic transition. During phase II and III, fruit abscission is reduced though in some species and under adverse environmental conditions ripe fruits may show pre-harvest fall.

Citrus fruits have two abscission zones. Flower and ovary abscission occurs between the branch and the fruit peduncle that becomes progressively inoperative during phase I during the first 8 weeks of fruit development. While the second abscission takes place in the calyx between the ovary wall and the nectary which is stimulated during June drop and at ripening (Iglesias *et al.*, 2007) thus fruit older than 8 weeks. Conversely, young fruits have the ability to abscise from the calyx. Matured fruits fail to abscise in between the branch and the fruit peduncle because of extensive lignification and secondary wall formation of abscission zone cells that surround the vascular tissue and inner cortex. Normally auxin delays abscission however the effect of auxin on abscission is associated to the endogenous hormonal balance that mostly changes as abscission proceeds. Marked physiological and biochemical changes take place during abscission of mature citrus fruit (Kazokas, 1997). Generally, a small percentage of fruits overcome the June drop and in general less than 1% reaches ripening.

2.4.1 Maturation and Ripening of Non-Climacteric Fruits

The fruit organs responsible for ripening represent the terminal stage of development where the matured seeds are released. In the dehiscent fruit, the ripening process is facilitated by senescence of the mature carpel tissue after which separation of the valves at an abscission cell layer is formed between the valve-replum boundaries (Ferrandiz *et al*., 2000; Roeder *et al*., 2003).

The specific biochemical processes that result in ripening phenomena that varies among species and the changes include: colour modification through the chlorophyll alteration, carotenoid and flavonoid accumulation, textural modification through alteration of cell wall and cell turgor structure and/or metabolism, modification of sugars, acids, and volatile profiles that affect nutritional quality, flavor and aroma, as well as enhancing susceptibility to opportunistic pathogens associated with breakdown of cell wall integrity (Giovannoni, 2004).

During citrus non-climacteric ripening process, active growth in fruits slows down and metabolism moves to sugar fruit demand to integrate biochemical and physiological changes that render an edible organ. Ethylene production and sensitivity are low in citrus ripe fruits, respiration is attenuated, change in texture and composition proceed (Iglesias *et al.*, 2007). Iglesias *et al.* (2007) reported that there is no evidence of specific hormone that controls the process of ripening. External and internal ripening coincide and so peel and pulp behave as separate organs and are considered as different physiological processes

2.4.2 External Fruit Ripening of Citrus

The external citrus fruit ripening process is dependent on the chloroplast conversion to chromoplasts and this includes the chlorophylls progressive loss and carotenoids gain, changing peel colour from green to orange or yellow or red (Iglesias *et al*., 2007). The changes are comparable to the senescence of vegetative chlorophyllous tissues and the fruits are influenced by environmental conditions and nutrient availability (Iglesias *et al*., 2001). Before carotenoid build-up, transition occurs from carotenoids of the photosynthetic chloroplast to the intensely colored carotenoids of the chromoplast (Gross, 1987). Chromoplast biogenesis is of particular interest and agronomical relevance, even from fully differentiated chromoplasts (Goldschmidt, 1988). The regulatory mechanisms that involve phytohormone control are involved in chloroplast transformation (Ben-Arie *et al*., 1995; Guis *et al*., 1997). Mature fruits release low amounts of ethylene though exogenous ethylene accelerate color break through both chlorophyll degradation and carotenoid deposition. Exogenous ethylene accelerates chlorophyll disappearance and increases chlorophyllase activity (Azuma *et al*., 1999; Fujii *et al*., 2007). The color break of fruit is controlled by nutrients (Iglesias *et al*., 2001).

2.4.3 Citrus Fruit Drop

The fruit drop pronounced stages:

(i) occurs after fruit-set at the marble stage and lasts a month after flowering, called post-set drop,

(ii) occurs at the beginning of hot weather between May to June, called June drop. According to Stösser (2002) the main cause of June drop is some kind of difficulty in the translocation of nutrients but at this stage of fruit development the requirement of organic substances is culminating.

(iii) occurs during ripening period, which lasts from August to December and sometimes January depending on the variety and it is called pre-harvest fruit drop. Higher temperature, excess or deficiency of soil moisture, lack of nutrients such as zinc, phosphorus and potassium, as well as infection of fungal diseases like anthracnose and black spot disease are primary factors responsible for pre-harvest fruit drop (Jeyakumar,

2011). In sour cherry, pre-harvest fruit drop is rare, only at excessively heavy fruit load (Soltész, 2003).After the cleaning or post-set fruit drop, the influence of temperature on the rate of fruit set diminishes and the significance of nutrition and water supply in the maintenance of fruits increases (Soltész, 2002).

2.5 Effect of N, P, K and Zn on Citrus Pre-Harvest Fruit Drop

Ashraf *et al.* (2012) reported that inadequate nutrient management in various citrus farms where soil and leaf analysis showed deficiency of Zn resulted in yield losses of kinnow. The fruit trees were pre-treated with NPK then K (K_2SO_4 solution), Zinc ($ZnSO_4$ solution) and salicylic acid were sprayed at three different stages, (i.e. the onset of flush of leaves or flowers, fruit formation and colour initiation on matured fruit). Fruit drop was reduced by spray of Zn, K, SA or Zn+K but three foliar sprays of salicylic acid + Zn and K reduced the citrus fruit drop by thirty percent.

Sajid *et al.* (2010) found that foliar applications of Zn ($ZnSO_4.7H_2O$), B (H_3BO_3) as well as, combination of Zn and B did not influence fruit set and fruit drop significantly.

2.6 Physiological Roles of Soil-Plant Nutrients on Citrus

2.6.1 Nitrogen (N)

Nitrogen is primary importance in production of citrus. It is very influential on tree growth and production of fruit than all the other elements. Growth is restricted and the foliage turns pale green or yellow when N is insufficient. When N is supplied to bearing trees at inadequate rates for some time, the trees adjust by shedding old leaves and translocating available N into the new leaves leading to a thin canopy. The green colour of the remaining leaves may be normal but the canopy is hollow inside. In cases of persistent N shortage, defoliation, fruit drop and shoot death occur (Obreza *et al*., 2011).

In addition, plants deficient in nitrogen are prone to pest and disease attack (Kahl, 2004). Also, deficiency of N causes early maturing in some crops which reduce yield and its quality (Uchida, 2000). Proteins are not synthesized though there can be abundance of available nitrogen (N) when plants are deficient in potassium (Spectrum Analytic Inc., 2016).

2.6.2 Phosphorous

An adequate Phosphorus (P) supply is necessary for new cells development. Plant cells accumulate nutrients at higher concentrations than present in the soil solution that surrounds them. Phosphorous is highly reactive element and it does not exist in the elemental form in the soil. The majority of P in most soil is in the insoluble forms and unavailable to plants. Plant roots take up all P in either the primary or secondary orthophosphate anion. Primary orthophosphate is the form that is dominant in acid soils and is taken up about 10 times as readily as the secondary orthophosphate form (Spectrum Analytic Inc., 2016).

As moisture stress increases, there is decrease in P availability and uptake. Phosphorus uptake is higher when in combination with ammonium N. In fertile soil a significant portion of the total P is in soluble forms and act as a reserve to replenish the pool of soluble P as it is exhausted by other organisms (Spectrum Analytic Inc., 2016). The effects of P deficiency on plant growth include delay of maturity, reduction of forage quality, fruit as well as vegetable and decrease disease resistance of plants (Osman, 2012). Low supply of phosphorus result in reduction in plant growth, limited flower development with reduced fruit set and fruit yield. Phosphorus deficiency occurs in areas of high rainfall due to leaching and erosion. Phosphorus availability is reduced in calcareous soils (Zekri and Obreza, 2015). Phosphorus is a primary plant nutrient required by citrus plant for sustaining long term performance. It has been demonstrated that limited P availability of low fertility in tropical soils predominantly impairs citrus production (Quaggio *et al.*, 2004).

2.6.3 Potassium (K)

Adequate level of potassium increases crop yields and reduces production losses, increases drought or stress resistance, activates enzyme systems, maintains turgor; decreases water loss, helps in photosynthesis and food formation. Potassium promotes translocation of sugars and starch, builds cellulose, reduces lodging as well as helping to retard crop diseases. Adequate K supply helps to keep all of the processes and transportation systems to function adequately. Potassium also plays significant role in the water and nutrients transport throughout the plant. When K supply is reduced then there is depression in translocation of nitrates, phosphates, calcium, magnesium and amino acids. Potassium is not part of plants chemical structure though, it plays important roles in development of a plant. Plants with insufficient K supply are more susceptible to water stress. Potassium accumulation in plant roots results to gradient of osmotic pressure that draws water into the roots. Again, plant's transport system uses energy in the form of ATP, so photosynthesis and ATP synthesis are reduced. However, plant respiration proliferates and leads to slower growth and development. ATP is less available and the transport system is broken down. Photosynthates build up in the leaves and the rate of photosynthesis is minimized. The role of K in phloem and xylem transport is in conjunction with enzymes and plant growth hormones but adequate supply of K is essential to efficient operation of these systems. The enzyme that is responsible for synthesis of starch is stimulated by K. Moreover, when there is insufficient K, the level of starch declines while soluble carbohydrates and N compounds accumulate (Better Crops, 1998).

Citrus fruits remove large amounts of K as compared with other micro and macro nutrients. Potassium moves from leaves to fruit and seeds as they develop. It is essential for several basic physiological roles. Potassium is important in fruit formation and improves fruit size, flavor, and color. It helps reduce the influence of adverse weather conditions like drought or water stress, cold, and flooding. Potassium influences many enzymatic reactions and is mostly associated with every major plant function (Zekri and Obreza, 2015).

2.6.4 Zinc (Zn)

Micronutrients deficiency causes more disorders than macronutrients in fruit crops, (Jeyakumar, 2011). Plant hormones metabolism of auxin (IAA) and tryptophan decrease in deficiency of zinc condition, leaf growth stops as a result. Zinc is essential for synthesis of tryptophan, which is a requisite for auxin formation (indole acetic acid synthesis) therefore amount of auxin decreases by zinc deficiency (Pedler *et al*., 2000). Zinc availability is highly dependent on pH, it is higher in acidic soils and very low in alkaline soils. Deficiency occurs commonly on soils with alkaline or neutral pH that are sandy or have low organic matter content and usually high in available phosphorus (Nutrient Technologies, 2001). Generally, low concentration of plant growth regulator exerts a profound influence on physiological process of the plant. When zinc is deficient, terminal growth areas are first affected. When the available zinc is inadequate, plants will suffer from physiological stress as a result of the malfunction of several enzyme structures and other metabolic roles that zinc plays part (Alloway, 2008). Zn deficiency is the most diffused nutritional alteration in almost all the areas that citrus is produced. It is prevalent in sandy soils but frequently observed in soils that are alkaline and can be intensified by adequate level of phosphate and/or nitrogen fertilization (Boaretto *et al*., 2002).

Zinc absorption ability is minimized by high phosphorus exploitation and zinc in plant and soil has inverse interaction with phosphorus (Mousavi, 2011). When there is adequate phosphorus level available in the soil, it depresses zinc content of the plant.

Shoots and buds of plants with zinc deficiency results to low auxin content and might depress plant yield by as much as 50 % without producing any symptoms. Citrus is mostly prone to zinc deficiency than other fruit trees **(**Fageria and Stone, 2008).

Table 2.1 Guidelines for Interpretation of Citrus tree leaf analysis from nonfruiting twigs

Element	Deficiency	Low	Optimum	High	Excess
N (%)	< 2.2	2.2 - 2.3	2.5-2.7	2.8 - 3.0	> 3.0
P (%)	< 0.09	0.09 - 0.11	0.12 – 0.16	0.17 – 0.30	> 0.30
K (%)	< 0.7	0.7 – 1.1	1.2 – 1.7	1.8 – 2.4	> 2.4
Zn (mg/kg)	< 18	18 – 24	25 – 100	100- 300	> 300

Source: Koo *et al.*, 1984

2.6.5 Organic Matter and pH

Organic matter constitutes the soil's main reserve of nitrogen (N) and its importance in supplying N to crops is well known. Agronomic interpretation of organic matter according to Sbaraglia (2016), is <1.00 – very low; 1.1-2.00, low; 2.00- 3.00, medium; 3.00-4.00, high and >4.00, very high. pH plays a significant role in regulating the chemical and biological process in the soil. The availability of nutritive elements depends on its value. Microelements such as copper, zinc and manganese are particularly soluble in acidic environment and can become toxic (Sbaraglia, 2016). The availability of P, K, Fe and Cu is affected by pH and so it's advisable to maintain pH at 5.5 to 7.0 (Davies and Albrigo, 2006).

2.6.6 Interactions of Nutrients and Plant Diseases

Mineral imbalances lower the resistance of plants to diseases by establishing a more enabling situation for pathogens such as fungus, bacteria, nematodes and virus. Formation of mechanical barriers in plant tissues is affected by mineral nutrition. Plant nutrients level might affect plant vulnerability through plant metabolic changes that creates a favourable environment for occurrence of disease when there is nutrients deficiency. When plant is infected by pathogen, the plant's physiology (assimilation, translocation, uptake of mineral nutrient and utilization) is altered. Pathogens restrain nutrients in tissues and interfere with nutrients translocation by inducing nutrient toxicities. Pathogens use nutrients, reduce plants accessibility and thereby enhance the susceptibility of plants to infection. Essential nutrient shortages reduce the amount of the plant's natural antifungal compounds at the infection site. When the nitrogen level is out of balance with other nutrients, the synthesis of antifungal compounds decreases. Fungus and virus infections are controlled by adequate Zn fertilization or availability. Actually, N nutrition suppresses disease because adequate N availability and uptake is essential to the synthesis of various protein structures and enzymes needed in disease resistance and growth. Vigorously growing plant balances the most damaging effects of some diseases. Excess N stimulates excessive and weak vegetative growth that promotes the incidence of some diseases. However, excess N uptake create environments favourable for disease damage such as promoting juicy growth, thinner cell walls and plant density that can make plants more susceptible to infection. It has been reported that excess N delays maturity by extending the period available for disease infection and development (Spann and Schumann, 2013).

Spann and Schumann (2013) have revealed that adequate level of K reduces bacterial and fungal diseases (70%) and 60% of mites and insects infestation of the time. Generalization for K, *adequate supply mostly results in an increased resistance to attack by all parasites and pests.* Potassium deficiencies lower crops resistance to disease infections. Potassium changes the relationship of the host-parasite environment within the plant. When a plant becomes infected by a fungus, its natural defenses are triggered. The infection causes increased synthesis of fungus inhibiting phenolic compounds and flavonoids, at the site of infection and in other plant parts. These compounds production and transport is controlled in large part by the general nutrition of the plant (Spann and Schumann, 2013). But K is critical in this role and its shortage reduces the amount of the plants antifungal compounds at the site of infection. A high nitrogen-potassium ratio in a plant makes it more susceptible or vulnerable to disease infection (Spectrum Analytic Inc., 2016)

2.7 Pathogens Associated with Citrus Pre-harvest Fruit Drop

Many diseases or pathogens have been associated with citrus pre-harvest drop and examples are *Alternaria alternate, Phytophthora* species, *Pseudocercospora angolensis* and *Phyllosticta citricarpa*. Savita and Nagpal (2012) reported that *Phytophthora* spp. infects citrus fruit and cause brown rot that leads to fruit drop and postharvest decay. Brentu *et al*. (2013) reported that *Pseudocercospora angolensis* on citrus fruits contribution to yield losses range between 50 to 90%. Fruits with *Pseudocercospora angolensis* infection cause the fruits to ripe prematurely and drop. Kuate *et al.* (2002) as well as Chung and Timmer (2009), reported that *Pseudocercospora angolensis* accounted for 50 to 100% loss/yield. Pazoti *et al*. (2005) reported that among diseases affecting the commercial citrus production, the citrus black spot (CBS) is considered to cause

substantial losses.

2.7.1 Citrus Black Spot (*Phyllosticta citricarpa*) - Origin and Distribution

Phyllosticta citricarpa originated from South East Asia collectively with its host, citrus (Smith *et al*., 1997). The asexual and sexual forms were first described in Australia as *Phoma citricarpa* and *Guignardia citricarpa* respectively from citrus leaf litter. The spermatial state is a *Leptodothiorella* though the species has not currently been defined (Baayen *et al*., 2002). Schubert *et al*. (2010) reported that the citrus black spot (CBS) pathogen occurs in the following citrus growing countries: Indonesia, Kenya, Argentina, Australia, Brazil, Ghana, India, United States of America (USA), Nigeria, Philippines, South Africa (SA), Swaziland, Taiwan and Zimbabwe.

2.7.2 Conditions that Favour Citrus Black Spot Establishment

Black spot disease is an essential citrus disease in tropical and sub-tropical regions. The disease has been found in almost all the rainfall areas in South Africa. The global distribution of CBS is restricted by specific climatic parameters; the main restrictive parameter is the alternating cold and wet conditions (Paul *et al*., 2005). Environmental conditions needed for successful incidence of prone citrus material consist of the availability of adequate moisture and comparatively high temperatures that range between 18 - 30°C for a minimum period of 15 hours (Kotze, 1981). These conditions mostly prevail in the rainfall zones. In South Africa, the critical infection duration occurs from October to January (Kotzé, 1996). The infection period usually starts and ends a month earlier or later and that depends on prevailing rainfall and average temperature.

Fruit remains susceptible from fruit set until five months later however leaves continue to be susceptible from development stages till the tenth month (Truter *et al*., 2004). The two types of spores are produced by the pathogen and can infect susceptible citrus material (Kotzé, 1996). Pycnidiospore of the anamorph is produced in pycnidia on symptomatic fruit, leaf litter with highly susceptible cultivar. In the presence of sufficient moisture, ascospores are forcefully released from pseudothecia to a height of 12 mm to be disseminated by air currents, while masses of gelatinous pycnidiospores ooze from pycnidia to be distributed by water. Viable ascospores and pycnidiospores land on young attached citrus fruit as well as leaves and usually lead to successful infection in favourable environmental conditions (Whiteside, 1965). After successful infection, the pathogen remains hidden in the fruit and on leaves for months as a small knot of mycelium growth occurs between the cuticle and epidermis. The latent period in fruit lasts till maturity of the fruit, though several factors concerning the host and environment usually influence symptom expression. Leaf infections remain latent until 36 months before leaf fall and under favourable conditions, production of pycnidio- and ascospores on the leaf litter (Kotze, 1996). The two main morphologically comparable *Phyllosticta* species occur on citrus, *P. citricarpa* causes black spot infections on citrus, and *Phyllosticta mangiferae*, non-pathogenic to citrus, causes simply symptomless infections that remains latent (Bonants *et al*., 2003; Baayen *et al*., 2002). The endophytic characteristics of the fungi on citrus previously caused misconception that isolates of *Phyllosticta* that are obtained from citrus was citrus pathogen called *Phyllosticta citricarpa*.

2.7.3 Symptoms of Citrus Black Spots on Fruits

Citrus black spot is caused by *Phyllosticta citricarpa* Aa and it signifies superficial surface fruit spots that are mostly unacceptable in citrus fresh fruit market. This poses a phyto-sanitary threat. Symptoms can develop on 90 % or more of fruits produced from unsprayed orchards with one to a high number of spots per fruit (Calavan, 1960). Fruit maturity and temperature stimulate the kinds of symptom that may appear. Citrus black spot diagnosis is very difficult except typical hard-spot symptoms that contain pycnidia of the fungus are observed on the fruits (Kotze, 1981). The symptoms that are widely recognized are hard, freckle and virulent spot as well as speckled blotch and cracked spot (De Goes *et al*., 2000). Virulent and hard spot might contain pycnidia in the lesions, though freckle spot can turn into speckled blotch and virulent spot may also turn into hard spot with time (Kotzé, 1981). Freckle spots on fruit are more unattractive as compared with hard spot alone (Kiely, 1948). After duration of hot weather, the fungal growth in the lesions rapidly increases as lesions briskly expand. Individual lesions combine to form a tearstain lesion comparable to melanose or develop extra into virulent spot (Baayen *et al*., 2002). These symptoms mostly appear following colour change from green to orange (Kotzé, 1981). Symptom expression of fruit that is matured is facilitated by high light intensity, rising temperatures, drought stress and plant vigour. Generally, an older tree develops more black spot than a younger tree. Suitable period of wetness, temperatures and inoculum must be present concurrently for infection to occur.

2.7.4 Economic Losses Attributed To Citrus Black Spots

The disease has brought hurdles to trade, due to phytosanitary risk linked with the export of fruit from areas with CBS positive production to the major international markets (EU and USA) (Baayen *et al*., 2002). The economic losses attributed to CBS consist of premature dropping of fruit in heavy infected farms, symptomatic fruit with low market value and high production costs as a result of extensive control measures that are usually undertaken (Kellerman and Kotzé, 1977). Citrus black spot causes total loss of the marketable crop in some citrus growing areas, and without effective CBS planned control methods, citrus production will not be feasible (Smith, 1996) or not profitable. The degree of post-harvest loss is not mostly apparent as latently infected, fruit without symptoms develop black spot symptoms while in transport and may be unacceptable upon arriving at destination (Kiely, 1948; Brodrick, 1969).

2.7.5 Control of Citrus Black Spot Disease

Dithiocarbamates were known in 1964 as preventive measure by applying zineb and later adding mancozeb (Kotzé, 1963). These were verified to be superior as compared to copper based fungicides (Kellerman and Kotzé, 1977) since it did not affect fruit colour development. Strobilurins were specified to be a good alternative for benomyl in orchards with known resistance of the citrus black spot pathogen to benomyl (Schutte *et al*., 1996; Tollig *et al*., 1996; Schutte *et al*., 2003). The strobilurins protect, cure, eradicate and provide lasting residual control of the disease and is recommended in addition with other fungicides such as copper or mancozeb to control citrus black spot (Schutte *et al*., 2003; Miles *et al*., 2004).

When trees are in a poor condition, they are more prone to citrus black spot. This is because tree vigour can minimise the incidence of citrus black spot. However, the most important and less expensive approach in citrus black spot control is to use agronomic practices to minimise transmission; the sources of pycnidiospore inoculum removal; removal of matured infected fruits from the field that is late-hanging fruit before the new fruit sets season (Calavan, 1960; Kotzé, 1996). Probably, as any citrus plant that is nutritionally stressed increases the chances of CBS infection (http://america.pink/citrusblack-spot_1003068.html), adequate nutrients required for the citrus to increase its vigour and produce the required anti-fungal compounds against CBS should be applied.

2.8 Fruit Flies

2.8.1 Abundance and Distribution of Fruit Flies

Insects such as fruit fly have been named as one of the causes of citrus fruit drop. In Ghana reported that fruit fly; *Bactrocera invadens* and *Ceratitis capitata* cause substantial losses in fruits and vegetables (EMPAQ-MOFA, 2013). Appiah *et al.,* (2009), reported that the adults were present in the late valencia variety farms throughout the period of study. The two peaks were in September and April where adult fly abundance was apparent in citrus orchards. The dates fall within the major and minor citrus harvesting seasons, respectively during the mature stages of late valencia and red blood varieties. The two peaks of fruit fly abundance coincide with the period when most citrus fruits are ripening or ripe. The study shows the relationship between the abundance and the distribution of *Ceratitis capitata* and the fruiting of late valencia citrus. This presupposes that temperature and rainfall have significant effect that is a significant on pest population. The results validate the fact that the fruit fly populations are sustained by different citrus varieties, which mature at different periods annually. Adalton *et al*. (2004) worked on fruit fly infestation in citrus in the São Paulo, Brazil and the results showed that 90% of citrus samples were not infested by Mediterranean fruit fly. This species had been reported with emphasis as a serious pest of citrus in earlier studies conducted in the state of São Paulo by a number of researchers. The changing of status was attributed to the intensive urbanization process of the sampled region.

2.8.2 Farmers' Perception on Fruit Flies

Badii *et al*. (2012) reported that most farmers are aware of the fruit fly problem in Ghana and perceived it to be very serious. Majority of the farmers demonstrated poor knowledge in identification of fruit fly species of economic importance, especially *Bactrocera invadens*. Farmers are more knowledgeable with the economic impact of fruit flies than their direct damage on host fruits. This implies that their role in causing pre-harvest fruit drop might not have been recognized by farmers. This is to the extent that recommended fruit fly control strategies such as pheromone trapping, bait application, soil inoculation and biological control were unknown or inaccessible to farmers. They mostly use chemicals that are not recommended for the control of fruit flies with no consideration to the environment and health.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Area

The study was conducted in the three major citrus growing districts; Ahafo Ano South, Bekwai Municipal and Adansi North in the Ashanti Region of Ghana. These areas are Moist Semi-deciduous agro-ecological zone noted for cultivation of citrus, cocoa and other horticultural crops such as, tomato, garden egg, pepper and okra. Ahafo Ano South is located on longitude -1.96 W and latitude 6.92 N; Bekwai Municipal is located on 6.45 N and longitude of -1.58 W and also Adansi North district is located on Longitude -1.52 W and latitude 6.28 N (http://gha.geonamebase.com/node). The districts fall within a typical tropical region of Africa and they all experience high temperatures (22–35^{o} C) and high rainfall throughout the year.

The study areas were purposively selected in order to assess the required data or information from farmers and their various farms.

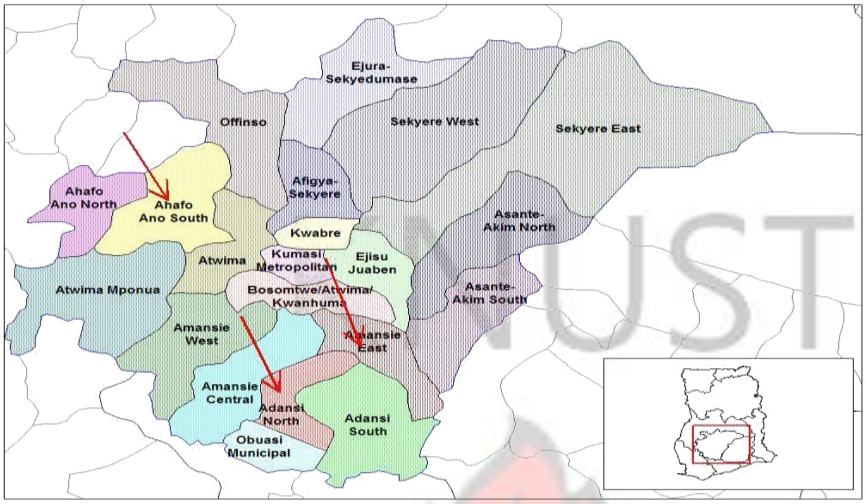


Figure 3.1 Map of Ashanti Region showing the major citrus growing districts for the study (arrows pointing to the districts visited)

3.2 Survey on Farmers' Perception on Causes of Citrus Pre-Harvest Drop and their Agronomic Practices

3.2.1 Study Design and Structure

Structured questionnaire was used to assess citrus farmers' perceptions on citrus preharvest fruit drop. After the questionnaire was pre -tested, data were collected from the selected respondents by visiting communities with citrus farmers. Comments with valid and reliable information were noted.

3.2.2 Sampling Size and Sampling Procedure

Snow-ball sampling technique was used to obtain citrus farmers in all the three major citrus growing areas. The communities that were chosen from each selected area was selected based on the availability of commercial citrus farmers (Badii *et al*., 2012). The questionnaire was administered to sixty (60) citrus famers with 24 farmers in Adansi North, 18 in Bekwai Municipal and 17 in Ahafo Ano South, (thus 20-30 % of citrus farmers' based organisation in the respective areas).

3.2.3 Data collection procedure

Based on the comments of the respondent during the pre-test, necessary changes were made on some aspects of the questionnaires. The respondents were approached individually either in their farms or homes. The purpose of the research was made know to the respondents after establishing initial rapport where they requested to volunteer for the study.

3.3 Estimation of Severity of Citrus Pre-Harvest Fruit Drop

Commercial citrus farms (8-16 years) were visited and an estimated one hectare (1 ha) was used in all the farms to count the number of fruits that dropped per tree in the major citrus harvesting season. The estimation was done from mid-August to mid-September for the red blood variety and mid-December to mid-January for late valencia. Grid method was used to select trees per variety for the counting or estimation of the dropped fruits. Sixty trees (60) per variety in each area were used. In all, 360 trees were used for the estimation of the mature fruits that dropped prior to harvesting from the selected districts.

3.4 Collection of Soil and Plant (Leaf and Fruit Peels) Samples for Laboratory Analysis

Observation and samples collection trips were made to five citrus orchards at five different communities in each selected district or municipal. In each orchard, grid method of sampling was used to collect samples in order to have a representation of field variability. From each farm, matured leaves (30 leaves per farm) from non-bearing terminals and twelve (12) soil cores/slice were taken to make a thoroughly mixed composite sample from 0-15 cm soil depths and taken for laboratory analysis from estimated one hectare size of the visited citrus farm. Again, twenty-four citrus fruit samples each for dropped and un-dropped fruits were also taken from twelve (12) trees at the sites for analysis (Owusu-Donkor, 2011) for each of the varieties. The plant and soil samples were sent to Soil Research Institute, Kumasi to determine the plant nutrients (N, P, K and Zn) and soil nutrient (Organic carbon, N, P, K and Zn) concentrations respectively in addition to pH (Obreza *et al*., 2011).

3.4.1 Plant Nutrients Analysis

Citrus fruits peels (dropped and normal or un-dropped) were collected for plant nutrient analysis. The plant materials were oven-dried, grinded and ashed before analysis. From this ground material, the nitrogen (N), phosphorus (P), potassium (K) and zinc (Zn) were determined. Levels of N were determined using the Micro-Kjeldahl method. Total phosphorus in solution was determined calorimetrically by the Vanadatephosphomolybdate method. The total K was determined by preparing an ash solution from the ashed peels then flame photometer was used to take the reading. Zn was determined using an atomic absorption spectrophotometer by preparing ash solution (Nartvaranant, 2012). Using the Micro-Kjeldahl method, the amount of N (%) was calculated as shown below:

$$\% \text{ N} = \frac{\text{Molarity of HCl} \times \text{titre value} \times 0.014 \times \text{vol.of extractant}}{\text{Weight of soil sample} \times \text{volume of aliquot}} \times 100$$

3.4.2 Soil Nutrients Analysis

Composite soil samples were collected at a depth (0-15 cm) to evaluate the trend of nutrient distribution in the soil in the selected orchards in the three various districts. The samples were taken to the laboratory for the determination of pH level as well as N, P, K and Zn concentrations respectively, % organic carbon (% O.C) and organic matter concentrations. Samples were air-dried, grounded, sieved and thoroughly mixed before analysis. A 1:1 soil:water ratio was used to determine pH with the help of pH meter. With available zinc, EDTA with ammonium acetate was used for extraction and was read with atomic absorption spectrophotometer. Wet combustion method of Walkley and

Black (1934) was used to determine % organic carbon and % organic matter. Where Organic carbon was calculated using the formula:

$$\%\ \text{Organic Carbon} = \frac{K_2Cr_2O_7\ \text{used} - \text{Titre value} \times 10}{\text{Titre value of the blank solution}} \times K$$

Where K is a constant (0.39)

Bray-1 solution was used to determine available phosphorus content of the soil. Concentrations of P in the mixture was then determined using the spectrophotometer. It was calculated by using the formula

$$\text{Available P} = \frac{X}{0.0878} * 7$$

Where X is the absorbance and 7 is the extraction ratio (i.e. 1:7, 5 g soil: 35 mls of Bray I solution).

3.5 Lesioned Fruits for Pathogen Isolation

Infected pre-harvest dropped citrus fruits were collected and taken to the Plant Pathology laboratory, Faculty of Agriculture, KNUST. The kind of pathogen possibly associated with citrus pre-harvest fruit drop of citrus was cultured, isolated and identified.

3.5.1 Chloramphenicol-amended Potato Dextrose Agar (CPDA) Preparation A 39 g of potato dextrose agar (PDA) was amended with 500 mg chloramphenicol in 500 ml of distilled water and topped up to 1000 ml in a beaker. The solution was stirred and transferred to volumetric flask and sterilized in an autoclave at a temperature of 121^{o}C with a pressure of 0.98 kg/cm^2. The prepared CPDA was autoclaved for 30 minutes and allowed to cool to about 45^{o}C before pouring about 20 ml into the sterilized petri dishes in the lamina flow cabinet. The poured CPDA was allowed to solidify before culturing the material.

3.5.2 Isolation and Identification of *Phyllosticta Citricarpa*

The lesions on fruit samples were cut with scapel, surface disinfected with 70 % ethanol for 30 seconds, sterilized with 2 % sodium hypochlorite for two minutes. This was followed with rinsing of the excised tissues twice with distilled water and blotted with filter paper. Prior to plating, lesions were excised carefully to remove any asymptomatic tissue. Cultures were placed near-ultraviolet light at 22^{o}C to facilitate the induction of pycnidia formation (IPPC, 2014)

3.6 Statistical Analysis

All the parameters from the analytical study were statistically analysed by using Genstat statistical package (version 11) at the probability level of 5 % using 2 x 3 factorial experiment arrangement in RCBD with first factor at 2 levels (varieties) and second factor at 3 levels (districts). Some of the data were transformed using square root and logarithm transformation, thus the count and percent data. Means were separated using least significant difference (lsd) and also relationship (r) analyses were conducted. Also, on the survey data, descriptive analysis was done with the help of the Statistical Package for Social Sciences (SPSS).

CHAPTER FOUR

RESULTS

4.1 Farmers' Perception on Causes and Management Practices of Citrus Preharvest Fruit Drop

Table 4.1 Farmers' Perception on the Causes of Pre-harvest Citrus fruit drop

Questions	**Respondents (%)**
Knowledge on pre-harvest fruit drop	
a) Yes b) No	100.0
Total	0.0
	100
Period pre-harvest fruit drop mostly occurs a. Aug/September-Dec/January	
(major)	20.0
b. March/April - May/June (minor)	10.0
c. Both Major and Minor	40.0
d. August – November	26.7
e. Don't Know	3.3
Total	**100**
Fruit flies cause pre-harvest fruit drop a. Yes	
b. No	95.0
Total	5.0
	100
What attracts fruit flies a. Dry season/favourable season	
b. Scent from flowers and scent/orange colour of mature fruits	13.3
c. Fruit fly infestation from previous year dropped fruits	40.0
d. Inappropriate farm management	10.0
e. Don't know	5.0
Total	31.7
	100
Damage caused on the fruits a. pierce the fruits and lay eggs	
b. don't know	88.3
Total	11.7
	100
Citrus black spot disease causes pre-harvest fruit drop	
a) Yes b) No	83.3
c) No response **Total**	11.7
	5.0
	100

4.1.1 Farmers' Perception on the Causes of Citrus Pre-Harvest Fruit Drop Farmers' perception on pre-harvest fruit drop was assessed and all (100 %) the farmers responded that they have knowledge of citrus fruit pre-harvest drop (table 4.1). About one-third (40 %) indicated that the dropping of matured fruits occurs in both major and minor seasons before the maturity index will be favourable for harvesting, thus Aug/September-Dec/January (major) and March/April - May/June (minor). This was followed by 26.7 % of farmers who are of the view that it occurs in between August and November. Only 3.3 % of the farmers said they don't know when the pre-harvest drop occurs.

Almost all the farmers (95 %) indicated that fruit flies contribute to the causes of the fruit drop. Some of farmers were of the view that the fruit flies are attracted by the scent from flowers and scent or orange colour of mature fruits (40 %) attracts this insect pest. Meanwhile 31.7 % said they do not know what attracts the fruit flies. Again, farmers' knowledge on the damage caused on the fruits by the fruit flies was assessed and 88.3 % indicated that they pierce the fruits and lay eggs whiles 11.7 % said they do not know the damage the fruit flies cause. Also, most of the farmers (83.3 %) had perception that citrus black spot disease is one of the causes of citrus pre-harvest fruit drop. They said citrus black spot disease appear and forces the fruits to ripen when the fruits have reached the mature size. This forced ripening is called *kyembedie* in local (twi) language.

Figure 4.1 Black spots symptoms causing forced ripening, known as '*kyembedie'* by farmers

Table 4.2 Control Measures of the Perceived Causes of Citrus Pre-harvest Fruit Drop by Farmers

Questions	Respondents (%)
Control of fruit flies a) Bait application and use of pheromone trap	28.3
b) Use of pheromone trap and insecticides	10.0
c) Spray poison or insecticide	28.3
d) No control	33.4
Total	**100**
Control of disease(s) a. Insecticides (poison)	11.7
b. Fungicides	11.7
c. Both insecticides and fungicides (mix them)	21.6
d. No control	55.0
Total	**100**
When chemicals are sprayed	
a. When black spot symptoms are seen	25
b. Flowering and fruit developing stages	16.7
c. No response	58.3
Total	**100**

4.1.2 Control Measures of the Perceived Causes of Citrus Pre-Harvest Drop by Farmers

Farmers' strategy in controlling fruit flies was assessed and 33.4 % indicated that nothing has been done yet to control the insect pest, 28.3 % use bait application whiles 28.3 % of citrus farmers use insecticides to control the fruit flies (Table 4.2). Again, farmers indicated that ‘black spot’ disease causes citrus pre-harvest drop and the agronomic practice(s) farmers undertake to control the disease they suspect was assessed and onehalf (55 %) reported that nothing has been done about it yet. Whiles 21.6 % mix both insecticides (poison) and fungicides to spray as their control measure, others (11.7 %) also indicated that they either use insecticides or fungicides. Further question was asked on when the chemicals are applied and 58 % gave no response. One-fourth (25 %) of the farmers spray chemicals when the symptoms are seen but 16.7 % indicated that spraying is done during flowering and fruit developing stages before symptoms are seen.

Table 4.3 Demography and Farmers’ Routine Agronomic Practices

Percentage (%)	**Percentage (%)**
Sex	
a) Male	80
b) female	20
Total	**100**
Level of education	
i. No formal education ii.	26.7
Primary iii.	15.0
JHS/Middle school iv.	36.7
Secondary v. Tertiary	13.3
Total	8.0
	100
Citrus farming experience	
a. 1 – 5 years	10.0
b. 6 – 10 years	51.7
c. 11 – 15 years	21.7 10.0
d. 16 – 20 years	6.7
e. Above 20 years	**100**
Total	
Frequency of weed control	
a) Once a year	6.7 56.7
b) Twice a year	30.0
c) Three times a year	6.7
d) Not at all **Total**	**100**

Pruning	
a) Yes	65.0
b) No	35.0
Total	**100**
Fertilizer application	
a) Yes	33.3
b) No	66.7
Total	**100**

4.1.3 Demography and Farmers' Agronomic Practices

About one-third of the farmers (36.7 %) had JHS/Middle school education while 26.7 % had no formal education. Majority of the citrus farmers (51.7 %) had been in citrus production from 6 - 10 years followed by those in their 11th – 15th year (21.7 %) of production but those who have been cultivating citrus for 20 years and above are very few (6.7 %), (Table 4.3). More than half of the farmers (56.7 %) indicated that they control weeds in their orchards twice in a year followed by 30 % clearing or controlling weeds three times a year. A minority of the farmers (6.7 %) either control weeds in their citrus farms once in a year or not at all. On pruning, two-thirds (65 %) of farmers said they prune their citrus trees and the remaining indicated that they do not prune their orchards. Farmers were asked if they fertilize their citrus farms since the trees started bearing fruits, two-thirds (66.7 %) responded no and one-third (33.3 %) indicated that they do fertilize.

4.2 Severity of Citrus Pre-harvest Fruit Drop in the Major Citrus Growing Areas

Figure 4.2 Severity of citrus pre-harvest fruit drop at a farm

Table 4.4 Means and Ranges of Citrus Pre-Harvest Fruit Drop from the Selected Areas

Areas	Red Blood			Late Valencia		
	Mean	**Range**	**Standard Error**	**Mean**	**Range**	**Standard Error**
Bekwai Municipal	167	18 - 493	14.32	87	19 - 291	9.22
Ahahfo Ano South	228	33 - 595	18.24	108	13 - 254	7.67
Adansi North	138	10 - 349	14.00	61	12 - 154	4.59

Ahafo Ano South had the highest means of citrus pre-harvest fruit drop for both varieties (Table 4.4). Thus, the mean for red blood variety was 228 with a range of 33 – 595 whilst the highest mean of late valencia was 108 from a range of 13 – 254. Adansi North had the lowest means and ranges of late valencia (61, 12 - 154) and red blood (138, 10 - 349) varieties. The range indicates the set of values from which the mean was obtained.

Table 4.5 Comparison of Means of Varieties and Districts of Citrus Pre-harvest

Drop

Variety	Means	District	Means
Red Blood	168a	**Ahafo Ano South**	109a
Late Valencia	78b	**Bekwai Municipal**	110a
		Adansi North	96a
	CV % = 34.5		

CV = coefficient of variation

The means indicate that red blood (168) was significantly different from late valencia (78) at 5% (< 0.05). However, the districts were not significantly different from each other ($p \geq 0.05$), (Table 4.5).

4.3 Soil and Plant Nutrients Levels from the Three Major Citrus Growing Areas

Table 4.6 Soil Analysis from the Selected Areas

Areas	N (%)	P (%)	K (mg/kg)	Zn (mg/kg)	% O. C.	% O. M	pH
Ahafo Ano South	0.13	5.33			1.65	2.84	6.16
Bekwai Municipal	0.13	4.91	48.40	11.39	1.60	2.76	5.73
Adansi North	0.16	4.00	41.50	14.59	1.86	2.17	5.78
			89.80	13.22			

4.3.1 Soil Analysis from the Selected Areas

Adansi North had the highest mean of 0.16 % nitrogen (N) whiles Ahafo Ano South had the highest mean of 5.33 % phosphorus (P) followed by Bekwai Municipal (4.91 %). Again, Ahafo Ano South had the highest mean of 89.8 mg/kg for

Potassium (K) but Adansi North had the lowest K of 41.5 mg/kg. The highest mean of 1.86 % organic carbon was at Adansi North whiles Bekwai Municipal had the lowest level of 1.60 % but Ahafo Ano South had the highest level of 2.84 % organic matter and the lowest level of 2.17 % at Adansi North. The highest pH of 6.16 was recorded for soils at Ahafo Ano South and the minimum (5.73) was obtained at Bekwai Municipal (Table 4.6). Zinc was high at Adansi North (14.59 mg/kg) and lowest at Bekwai Municipal (11.39 mg/kg).

Table 4.7 Plant Analysis from the Selected Areas

Farm	Late Valencia N (%)	P (%)	K (%)	Zn (mg/kg)	Red Blood N (%)	P (%)	K (%)	Zn (mg/kg)
				Leaf Analysis				
Ahafo Ano South	3.11	0.17	0.61	4.70	2.84	0.18	0.60	5.06
Bekwai Municipal	2.17	0.13	0.49	7.02	2.41	0.15	0.41	6.02
Adansi North	2.31	0.13	0.41	7.86	2.49	0.17	0.45	5.52
				Un-dropped Fruit Peels				
Ahafo Ano South	4.08	0.09	0.56	2.36	4.74	0.08	0.58	2.82
Bekwai Municipal	2.79	0.10	0.38	4.24	2.73	0.07	0.40	4.62
Adansi North	2.60	0.08	0.43	3.98	2.85	0.08	0.49	5.32
				Dropped Fruit Peels				
Ahafo Ano South	3.45	0.09	0.52	2.84	4.09	0.09	0.48	2.82
Bekwai Municipal	2.91	0.08	0.45	4.34	2.88	0.09	0.42	6.18
Adansi North	2.88	0.08	0.35	5.66	2.58	0.09	0.44	3.68

4.3.2 Plant Analysis from the Selected Areas for Late Valencia Variety

Ahafo Ano South had the highest level of nitrogen (3.11 %), phosphorus (0.17 %), potassium (0.61) but the lowest level of zinc (4.70 mg/kg), (Table 4.7). Bekwai

Municipal (BM) had the lowest level of nitrogen (2.17 %) and phosphorus (0.13 %). At Adansi North, there was high level of zinc (7.86 mg/kg) and low level of potassium (0.41 %) from the leaf analysis. From the un-dropped fruits peels, Bekwai Municipal (BM) had the highest level (0.10 %) of phosphorus whilst Adansi North had the lowest level (0.08 %). Potassium was high (0.56 %) at Ahafo Ano South but low (0.38 %) at Bekwai Municipal. In addition, Bekwai Municipal had the highest level of 4.24 mg/kg of zinc from the un-dropped fruits peels.

The order of level of nutrients from the leaf analysis follows similar pattern in the dropped fruit peels. Thus, Ahafo Ano South had the highest level of phosphorus (0.09 %), potassium (0.52 %) but the lowest level of zinc (2.84 mg/kg) whilst Bekwai Municipal and Adansi North had the lowest level of phosphorus (0.08 %) and potassium (0.35 mg/kg) in that order.

4.3.3 Plant Analysis from the Selected Areas for Red blood variety

Ahafo Ano South had the highest level of phosphorus (0.18 %), potassium (0.60 %) but the lowest level of zinc (5.06 mg/kg). Whilst Bekwai Municipal (BM) had the lowest level of phosphorus (0.15 %), potassium (0.41 %) and the highest level of zinc (6.02 mg/kg) from the leaf. With the un-dropped fruit peels whilst Adansi North had the highest level of phosphorus (0.09 %) and zinc (5.32 mg/kg). Ahafo Ano South had the highest level of potassium (0.58 %). Bekwai Municipal had the least level of P (0.07 %) and K (0.40 %) whilst AAS had the lowest level of zinc (2.82 mg/kg). Bekwai Municipal had the highest levels of phosphorus (0.09 %) and zinc (6.18 mg/kg). Ahafo Ano South had the highest level of potassium (0.48 %) and lowest level of phosphorus (0.08 %) and zinc (2.82 mg/kg) from the dropped fruit peels.

Table 4.8 Comparison of Plant Nutrients Means among the Selected Areas

Areas	N (%)	P (%)	K (%)	Zn (mg/kg)
		Leaf Analysis		
Ahafo Ano South	2.98a	0.17a	0.60a	4.88b
Bekwai Municipal	2.29a	0.14b	0.45b	6.52a
Adansi North	2.40a	0.15ab	0.42b	6.69a
CV %	17.3	16.1	20.5	12.1
		Un-dropped fruit peels		
Ahafo Ano South	4.41a	0.09a	0.54a	2.59a
Bekwai Municipal	2.67b	0.08a	0.39a	4.43a
Adansi North	2.82b	0.10a	0.46a	4.65a
CV %	10.00	23.10	34.60	23.90
		Dropped fruit peels		
Ahafo Ano South	3.77a	0.09a	0.50a	2.83a
Bekwai Municipal	2.75b	0.08a	0.44a	5.26a
Adansi North	2.79b	0.08a	0.40a	4.67a
CV %	9.8	23.3	28.8	25.4

Means with the same letter are not significantly different from each other

4.3.4 Comparison of Means from the Plant Analysis among the Selected Areas

4.3.4.1 Nitrogen (N) Levels in Citrus Leaf and Fruit Peels

There was no significant difference from the leaf analysis among the selected areas but the fruit peels (dropped and un-dropped) had significant differences among them. Thus, Ahafo Ano South was significantly different from both Bekwai Municipal and Adansi

North (Table 4.8) for both dropped and un-dropped peels.

4.3.4.2 Phosphorus (P) Levels in Citrus Leaf and Fruit Peels

It was only the leaf analysis that was significantly different from each other among the districts whilst phosphorus levels in both dropped and un-dropped fruit peels level were not significantly different from each other. From the leaf analysis, Ahafo Ano South (0.17 %) was significantly different from Bekwai Municipal (0.14 %) but not from Adansi North (Table 4.8).

4.3.4.3 Potassium (K) Levels in Citrus Leaf and Fruit Peels

The level of potassium in both dropped and un-dropped fruit peels were not significantly different among the areas except from the leaf analysis. Ahafo Ano South (0.60 %) had the level K that was significant from Bekwai Municipal (BM) (0.45 %) and Adansi North (AN) (0.42 %), (Table 4.8).

4.3.4.4 Citrus Leaf and Fruit Peels of Zinc (Zn) Levels

Both dropped and un-dropped fruit peels were not significantly different from each other but there were significant differences among the areas from the leaf analysis. Thus, Bekwai Municipal (6.52 mg/kg) and Adansi North (6.69 mg/kg) were significant over the Ahafo Ano South (4.88 mg/kg), (Table 4.8).

Table 4.9 Comparison of Plant Nutrients between the two varieties

	N (%)	P (%)	K (%)	Zn (mg/kg)
Variety		**Leaf Analysis**		
Late Valencia	2.53a	0.142a	0.501a	6.53a
Red Blood	2.58a	0.165a	0.486a	5.53a
CV (%)	17.3	17.0	25.1	27.6
		Un-dropped fruit peels		
Late Valencia	3.16a	0.091a	0.454a	3.53a

Red Blood	3.44a	0.076a	0.471a	4.25a
CV (%)	10.0	24.5	40.3	53.3
		Dropped fruit peels		
Late Valencia	2.02a	0.083a	0.441a	4.28a
Red Blood	3.19a	0.085a	0.447a	4.23a
CV (%)	9.80	24.80	33.60	55.7

Means with the same letter are not significantly different from each other

4.3.5 Comparison of Values from the Plant Analysis between the Varieties The nutritional levels of nitrogen, phosphorus, potassium and zinc in both late valencia and red blood varieties from leaf, dropped and un-dropped fruit peels were not significantly different from each other (Table 4.9).

4.3.6 Relationship (r) among Leaf Nutritional Levels from the Selected Areas

Table 4.10 Relationship (r) among leaf nutritional levels at Ahafo Ano South

	Nitrogen	Phosphorus	Potassium	Zinc
		Red Blood		
Nitrogen	1			
Phosphorus	-0.38	1		
Potassium	-0.85	-0.40	1	

Zinc	0.53	-0.22	0.28	1
		Late Valencia		
Nitrogen	1			
Phosphorus	-0.27	1		
Potassium	-0.18	-0.21	1	
Zinc	0.71	0.41	-0.14	1

4.3.6.1 Relationship (r) among leaf nutritional levels at Ahafo Ano South

At Ahafo Ano South, nitrogen associated with both potassium (-0.85) strongly and phosphorus (-0.38) inversely for red blood variety (Table 4.10). There was a weak relationship between nitrogen and potassium (-0.27) as well as between nitrogen and phosphorus (-0.18) negatively. for the late valencia but strongly relates with zinc (0.71) directly. Weak and moderate inverse relationships were observed between phosphorus and potassium for both late valencia and red blood respectively. Again, weak inverse relationship (-0.22) was observed between zinc and phosphorus for red blood but moderate relationship (0.41) existed for late valencia. Direct and inverse relationship occurred between potassium and zinc for red blood (0.28) and late valencia (-0.14) respectively, though they are not statistically significant.

Table 4.11 Relationship (r) Among Leaf Nutritional levels at Bekwai Municipal

	Nitrogen	**Phosphorus**	**Potassium**	**Zinc**
		Red Blood		
Nitrogen	1			
Phosphorus	-0.77	1		
Potassium	0.83	-0.62	1	
Zinc	-0.22	-0.06	-0.13	1

		Late Valencia		
Nitrogen	1			
Phosphorus	-0.60	1		
Potassium	-0.41	-0.22	1	
Zinc	-0.13	0.02	0.76	1

4.3.6.2 Relationship (r) among leaf nutritional levels at Bekwai Municipal

At Bekwai Municipal, nitrogen strongly relates with phosphorus (-0.77) and potassium (0.83) for red blood but inversely relates with phosphorus and potassium moderately for late valencia. There was no association between zinc and phosphorus for both late Valencia and red blood. The correlation between phosphorus and potassium was inversely moderate (-0.62) for red blood and negatively weak (-0.22) for late valencia. Strong relationship (0.76) existed between zinc and potassium directly for late valencia and weak inverse relationship (-0.13) for red blood variety (Table 4.11), though they were not statistically significant.

Table 4.12 Relationship (r) among leaf nutritional levels at Adansi North

	Nitrogen	**Phosphorus**	**Potassium**	**Zinc**
		Red Blood		
Nitrogen	1			
Phosphorus	0.31	1		
Potassium	0.52	-0.10	1	
Zinc	-0.67	-0.08	-0.14	1
		Late Valencia		

Nitrogen	1			
Phosphorus	-0.88	1		
Potassium	-0.50	0.32	1	
Zinc	-0.83	0.75	0.72	1

4.3.6.3 Relationship (r) among leaf nutritional levels at Adansi North

At Adansi North, nitrogen directly interacted with phosphorus (0.31) and potassium (0.52) but inversely with zinc (-0.67) for red blood variety (Table 4.12). With late valencia, there is inverse relationship between nitrogen and phosphorus (-0.88), potassium (-0.50) and zinc (-83). There was weak relationship between potassium and phosphorus for both red blood (-0.10) and late valencia (0.32). No relationship exists between phosphorus and zinc with red blood variety but for late valencia there was strong direct relationship (0.75). With potassium and zinc, weak inverse correlation (-0.14) was observed for red blood orange whilst strong direct association (0.72) among nutrients in late valencia, though they were not statistically significant.

Table 4.13 Leaf-Drop fruit Peel Zinc and Nitrogen Relationship (r) from the Selected Areas

Areas	Variety	Zn-Zn (r)	N-N (r)
Ahafo Ano South	Late Valencia	0.87	0.20
	Red Blood	0.63	-0.37
Bekwai Municipal	Late Valencia	0.15	0.62
	Red Blood	-0.31	0.51
Adansi North	Late Valencia	0.49	-0.39

Red Blood	-0.69	0.25

4.3.7 Leaf-Drop fruit Peel Zinc and Nitrogen Relationship (r) from the Selected Areas

With late valencia, there was direct relationship for both zinc (0.87) from leaf and fruit peels and nitrogen (0.20) at Ahafo Ano South whiles there was inverse association (-0.37) for nitrogen and moderately direct relationship for zinc (0.63) for red blood variety (Table 4.13). At Adansi North, late valencia variety had moderately direct relationship (0.49) for zinc and inverse association for nitrogen (-0.39) but red blood variety had strongly inverse association (-0.69) for zinc and weak association for leaf- drop fruit peel nitrogen (0.25).

4.4 Pathogen(s) Associated with Citrus Pre-harvest Fruits Drop

Figure 4.3 Black spot lesions on the dropped fruit

Table 4.14 Pathogens Identified from Cultured Black Spot lesions

Fungus/Variety	Frequency		
Late Valencia variety	**Ahafo Ano South (%)**	**Bekwai Municipal (%)**	**Adansi North (%)**
Phyllosticta citricarpa	55	30	30
Aspergillus niger	20	45	25
Aspergillus flavus	15	15	15
C. gloeosporoides	10	10	30
Red Blood variety			
Phyllosticta citricarpa	35	35	20
Aspergillus niger	15	20	25
Aspergillus flavus	25	25	30
C. gloeosporoides	25	20	25

4.4.1 Pathogens Identified from Cultured Citrus Black Spot lesions

The pathogens that were identified are *Phyllosticta citricarpa, Aspergillus niger, Aspergillus flavus* and *Collectotrichum gloeosporoides* for both late valencia and red blood oranges (Table 4.14). *Phyllosticta citricarpa* morphological growth was highest for both varieties, thus late valencia (55 %) and red blood (35 %) but *C. gloeosporoides* was the lowest (10 %) for late valencia at Ahafo Ano South and Bekwai Municipal and *Aspergillus niger* was the least (15 %) for red blood oranges.

Table 4.15 Percentages of Dropped Fruits with Citrus Black Spot Lesions and Fruit Flies Infestation

Areas	Citrus black spot lesions		Fruit flies infestation	
	Late Valencia (%)	**Red Blood (%)**	**Late Valencia (%)**	**Red Blood (%)**
Ahafo Ano South	81.63	87.11	18.36	12.42
Bekwai Municipal	74.64	92.18	25.14	7.92

Adansi North	89.64	84.50	10.94	16.12
Means	**81.97**	**87.93**	**18.15**	**12.15**

4.4.2 Percentages of Dropped Fruits with Citrus Black Spot Lesions and Fruit Flies Infestation

Adansi North (89.64 %) had the highest percentage of incidence of citrus black spot lesions and Bekwai Municipal (74.64 %) had the lowest percentage. Bekwai Municipal had the highest lesion of citrus black spot (92.18 %) for red blood whiles Adansi North also had the highest % for late valencia (89.64 %). The total mean of late valencia with citrus black spot lesions was 81.97 % and that of fruit flies infestation was 18.15 %. For red blood oranges, the total mean was 87.93 % and that of fruit flies infestation was 12.15 % whilst the highest level of incidence was 92.18 % at Bekwai Municipal (Table 4.15).

Table 4.16 Leaf Nutritional levels and Rate of Fruit Drop with Black Spot Lesions from the Selected Areas

Area	**Nutrients levels**				**Rate of Citrus Black Spot Lesions**	
	Late Valencia		**Red Blood**		**Late Valencia (%)**	**Red Blood (%)**
	K (%)	**Zn (mg/kg)**	**K (%)**	**Zn (mg/kg)**		
Ahafo Ano South	0.61	4.70	0.60	5.10	81.63	87.11
Bekwai Municipal	0.49	7.02	0.41	6.02	74.64	92.18

Adansi North	0.41	7.86	0.45	5.50	89.64	84.50

4.4.3 Leaf Nutrients levels and Rate of Fruit Drop with Black Spot Lesions from the Selected Areas

With late valencia variety, Adansi North had the highest level of zinc (7.86 mg/kg), lowest level of potassium (0.41 %) and the highest incidence of citrus black spot lesions (89.64 %), (Table 4.16). However, for red blood variety, Bekwai Municipal had the lowest level of potassium (0.41 %) concentration, the highest level of zinc (6.02 mg/kg) and the highest level of citrus black spot lesions (92.18 %) at Bekwai Municipal.

CHAPTER FIVE

DISCUSSION

5.1 Farmers' Perception on the Causes of Citrus Pre-harvest Fruit Drop

The fact that all the farmers who were interviewed in this study had knowledge of citrus pre-harvest fruit drop, suggests that probably citrus pre-harvest fruit drop is severe in their various orchards. This finding is in close association with reports that citrus preharvest fruit drop is a common problem (NPCS Board of consultants and Engineers, 2009). Citrus farmers have perception that late valencia variety drops more than the red blood variety. Their reason is that when a spell of dry season occurs and it rains again in August/September, where red blood has matured and/or ripened for harvesting, the dropping of the fruits is severe though much can be harvested than late valencia variety that starts dropping from September till the maturity period in January. Farmers' perception on the variety that drops more opposes the report of NPCS Board of consultants and

Engineers, (2009) which indicated that red blood variety drop more than late valencia of sweet orange. As reported by Appiah *et al.* (2009) a minority of citrus farmers indicated that the dropping of matured fruits occurs in both major and minor seasons before the maturity index will be favourable for harvesting, thus Aug/September to Dec/January (major) and March/April - May/June (minor). However, the perception of some farmers that fruit drop occurs in between August and November fits well with the early maturing varieties (Paul Yaw Agyei, Personal Communication, April 10, 2016) such as red blood variety.

Almost all the farmers (95 %) indicated that fruit flies contribute to the causes of citrus fruit pre-harvest drop. Their opinion agrees with Badii *et al*., (2012) who reported that farmers are mostly aware of the presence and economic importance of fruit flies. Some farmers were of the view that dry season/favourable season (13.3 %) attracts the fruit flies. *Ceratitis capitata* (med fly) for instance which is one of the fruit flies in Ghana (Appiah *et al*., 2009) has over 300 hosts and the evergreen nature of citrus trees (one of their hosts) attracts them to the orchards (Knowledge Master, 2016) to cause havoc to the fruits. Others reported that scent from flowers and scent or orange colour of mature fruits attracts this insect pest to the citrus farms (Enoch A. Osekere, Personal Communication, March 9, 2015). In addition, Appiah *et al.* (2009) reported the two peaks of adult fruit fly in Ghana and their abundance fall within the major (September) and minor (April) harvesting seasons during the mature stages of sweet orange varieties. These dates correspond with the period most citrus fruits ripe. Meanwhile 31.7 % of citrus farmers indicated that they did not know what attracts the fruit flies. This implies that no agronomic practice was undertaken to prevent the flies from reaching their threshold since they were not aware of what attracts them. Majority of farmers (88.3 %) had knowledge on the damage caused on the fruits by the fruit flies. Citrus farmers indicated that they pierce the fruits (ripe or unriped) and lay eggs (Department of Agriculture and Food, 2016) whiles 11.7 % said they did not know the damage the fruit flies cause. It can be confirmed that farmers were more conversant with direct damage symptoms on host fruits as well as their economic impact (Badii *et al*., 2012). Farmers' awareness of what attracts the fruit flies and damage on the fruits indicates when to undertake the recommended control measures in addition to sanitation in farms.

Also, majority of farmers' had perception that diseases were one of the causes of citrus pre-harvest fruit drop. They indicated that, 'black spot' symptoms were usually seen on the matured fruits which force the fruits to ripe. According to the farmers, the force ripened fruits may fully develop the orange colour though may not have attained edible maturity. The citrus farmers were of the view that when the black spot disease occurs on a tree, the yield will be high in the first year of occurrence then severe die back follows in the second year and by the fourth year, the tree will be on the path of dying. It has been found that citrus trees that are of low vigour (nutritionally stressed) are more susceptible to citrus black spot (Kellerman, 1975) or increases the chances of CBS infection. USDA (2010) reported that symptom expression on mature fruit is hastened by rising temperatures, high light intensity and drought stress and low tree vigour.

5.1.1 Control Measures of the Perceived Causes of Citrus Pre-Harvest Drop by Farmers

The one-third of citrus famers indicated that fruit flies were not controlled possibly that the farmers are either unaware or have no access to recommended fruit fly control strategies (Baadi *et al*., 2012) such as pheromone trap, bait application, soil inoculation and biological control (EMQAP-MOFA, 2013). Some of the farmers indicated that they combined bait application and pheromone trap in their orchards and they claimed both are very effective. In addition, other citrus farmers combined pheromone trap and insecticides as well, whereas the remaining used insecticides only. The farmers claimed that the recommended insecticides to control fruit flies are not known or not accessible. This assertion affirms Baadi *et al*. (2012) report that 72 % of farmers apply chemicals that are not recommended for the control of fruit flies.

Moreover, one-half of farmers did not undertake any measure to control 'black spot' disease they suspected to cause pre-harvest fruit drop indicated. Though, farmers were aware that 'black spot' disease forces the fruits to develop the orange colour whiles not matured for market or consumption and drop before harvesting, some of the farmers claimed the cost of chemicals to control the disease is expensive and decreases their returns. Citrus farmers (21.6 %) control citrus black spots by mixing both fungicides and insecticides to spray as their control measure to reduce citrus black spot disease are not likely to have reasonable efficacy. For instance, Nault *et al*. (2011) reported that mixing fungicides and insecticides in a tank is efficient because it saves time and fuel by pulling the sprayer through the field once instead of twice. However, this tank mixing practice may reduce the efficacy of either of the chemicals. Again, frequent application of fungicides and insecticides mix could aggravate or intensify resistance development.

In addition, the farmers who used fungicides only complained that they do not get or know the recommended chemical or fungicides to purchase and use. Others who know of the recommended fungicides, mancozeb (Kellerman and Kotzé, 1977; Schutte *et al*., 2003) did not get the chemical at appropriate time to use. Though, Strobilurins is commended in combination with other fungicides such as mancozeb or copper to control CBS (Schutte *et al*., 2003; Miles *et al*., 2004).

The citrus farmers who sprayed chemical(s) when the black spot symptoms were seen did not know either they spray curative or preventive fungicides. Farmers who indicated that chemicals were sprayed during the flowering and fruit developing stages before maturing stage were likely to get better results if the recommended fungicides and cultural practices were done appropriately. This is because the fruit remains susceptible to infection from fruit set up to 5-6 months later, (Truter *et al*., 2004) and so spraying mancozeb for instance will serve as a preventive measure to minimize the citrus black spot infection in the orchard.

5.1.2 Demography and Farmers' Agronomic/Management Practices

Citrus pre-harvest fruit drop has on a larger scale increased cost of production, minimize profit and has been observed that some citrus farmers have abandoned their farms, cut down their citrus orchard to cultivate cocoa and other crops. In ascertaining the agronomic practices undertaken by these farmers to minimize the citrus pre-harvest fruit drop, it was realized that about 86.7 % controlled weeds in their orchards twice three times a year. It is very relevant that most farmers control weeds regularly in their sweet orange orchards. This is because weeds compete with citrus trees for nutrients, water and light penetration which tend to have effect on yield and fruit quality (Davies and Albrigo, 2006; UC-IPM, 2016). Although, two-thirds (65 %) of farmers reported that they prune their orange trees, they categorically indicated that only dead parts are removed when weeding without considering overgrown parts, sunlight interception, alternate bearing and following the routine years of pruning citrus **(**Kelly and Wright, 2010). Generally, some of the farmers indicated that they did not know the importance of pruning to the citrus tree. This suggests that there can be high moisture that may favour insects and pathogens survival even in adverse weather conditions to cause damage to the crop. Khalid *et al.* (2013) reported that in a crowded orchard, disease and pests can spread quickly. Again, when citrus plant is not pruned appropriately or at all and the plant is stressed (biotic or abiotic) the plant will shed off their leaves and fruits as a natural defensive or survival mechanism (Zekri and Obreza, 2015). In addition, proper pruning enhances the tolerance of most stresses and aid to maintain the most efficient balance between vegetative growths and fruiting. Pruning ensures efficient water use and increases the conversion of available plant nutrients. Appropriate pruning program helps in controlling pests and diseases in citrus orchard (Khalid *et al.,* 2013).

Citrus fruits are known to remove essential amounts of N, P, and K as well as, other micronutrients in a season since sweet orange is known to be heavy feeder of nutrients. They are necessary for basic physiological functions such as synthesis of cell division, sugars and starch, organic acids neutralization and growth (Zekri and Obreza, 2015). Majority of citrus farmers did not fertilize their farms because they have perception that when they fertilize their citrus farms, it may reduce the life span of their farms; it may also reduce the shelf life of the harvested fruits but it is (Agnes Nyomora, Personal Communication, March 31, 2016) explained that fertilizer reduces the shelf life only when applied in excess. Again, the cost that is involved in fertilizer and its application is expensive. This implies that in a situation where there is insufficient nutrients in the soil, there can be delayed maturity, reduced quality of fruit, shedding of leaves and fruits as well as decreased crop disease resistance (Better Crops, 1998). Deficiency of P for instance appear on older leaves first, then they lose their deep green colour then leaves and fruits are shed prematurely before normal shedding or harvesting time (Zekri and Obreza, 2015). Sufficiently nourished citrus trees grow stronger and have better tolerance to pests and stresses more consistently than when deficient. Limited availability of nutrients in tropical soils predominantly impairs citrus production (Quaggio *et al.,* 2004). Since, citrus has been reported to be high feeders, regular (twice a year) fertilization for optimum performance

("Fertilizing and Pruning", 2016) is recommended. As most farmers refuse to fertilize their farms, the vigour of the citrus trees' is likely to be affected.

5.2 Severity of Citrus Pre-harvest Fruit Drop in the Three Major Citrus Growing Areas in Ashanti Region

Ahafo Ano South had the highest mean dropped fruits prior to harvest for both late valencia (228) and red blood (109) varieties than the other study areas, though they were not statistically significant difference. Early maturing varieties seem to be more prone to fruit drop than late maturing varieties. This is because Albrigo (2013) similarly reported on citrus pre-harvest fruit drop and early maturing variety (eg. red blood) had higher mean dropped fruit than late maturing varieties (eg. late valencia). This report agrees with NPCS Board of Consultants and Engineer (2009) who stated that malta blood red or red blood varieties are more prone to pre-harvest fruit drop than late valencia. Ahafo Ano South had the highest mean of dropped fruits for both varieties probably because of their faulty cultural practices such as no fertilizer application, inappropriate or no pruning as well as stresses such as nutrients deficiency and pathological infection.

The ability of trees to hold fruit is affected or influenced by the nature of the climate during and after maturity ("The Citrus Industry", 2016). Morton (1987) reported that red blood variety are commonly cultivated in the Mediterranean (temperature range 1^{o} to $38^{o}C$) area especially in Italy, Algeria, Tunisia, Morocco and Pakistan but grown very little in Florida where the red coloration only develops during cold weather periods. He indicated that late valencia is the most satisfactory orange for the tropics, even though it may not develop full orange colour in warm regions and added that fruits can remain on the tree for 6 months after maturity under favourable conditions only if it is not damaged by fruit fly or black spot. However, Davies and Albrigo (2006) and Morton (1987) have indicated that unfavourable climatic conditions enhances the drop rate to be severe due to climatic influence on some physio-chemical processes the plant or fruit undergoes. For instance, Butelli *et al*. (2012) reported that anthocyanin pigments that provide the blood colour are not produced in significant amounts unless the fruit is open to cold conditions during its synthesis. They indicated that poor anthocyanin production means no cold exposure and the loss of the entire crop. Davies and Albrigo (2006) reported that higher temperature accelerates growth rate and physiological developments of plant. This is because formation of anthocyanins is dependent on maturity and cool temperature according to Meredith and Young (1969) while Lo Piero *et al*, (2005) are of the view that induction of anthocyanins is induced by UVB radiation, cold temperature (Mediterranean climate) and low content of phosphorus content in the soil. Although, Borochov-Neori *et al*. (2011) reported that anthocyanin accumulation change in reverse to the temperatures of the season. Anthocyanins (monoglucosylated) prevail at cooler temperatures and subside during seasonal warming with a concomitant increase in diglucoside proportion. Essentially, Webb (2014) reported that anthocyanins in plants serve as protective mechanism against stress in the environment. Anthocyanins are believed to provide resistance against environmental hazards such as protecting tissues from photo-inhibition caused by high level of visible light and from oxidative damage or stress (Stintzing and Carle, 2004). Furthermore, Kimbell (1999) reported that anthocyanins are subject to physiochemical degradation both *vivo* and *in vitro* within an unfavourable environment. This shows that unfavourable climatic or environmental condition compels red blood orange to drop more than late valencia.

The two main causes of citrus pre-harvest fruit drop are physiological and pathological causes (NPCS Board of Consultants and Engineers, 2009). These two causes of preharvest fruit drop are mainly influenced by climate ("The Citrus Industry", 2016), variety cultivated and cultural practices such as weed control, pruning and fertilizer application etc. When the cultural or agronomic practices of citrus farmers were assessed, weed control was the only practice done regularly by most farmers without appropriate pruning, fertilizer application and disease control measures. Therefore, inconsistent cultural practices might have contributed to the high level of dropping of sweet orange in the selected area especially Ahafo Ano South. Maintenance culture of these farmers and unfavourable climate might have caused the increase in the severity of pre-harvest fruit drop in their farms. This is because when the citrus trees are nutritionally or physiologically stressed for instance, it sheds it leaves and fruits as their physiological defensive mechanism (Zekri and Obreza, 2015). Furthermore, if soil and plant nutrients are insufficient or below the optimum requirement of the tree plant, the required antifungal or anti-bacterial compounds will not be adequately produced to help the plants' resistance to either bacterial or fungal infections (Spectrum Analytic Inc., 2016). Furthermore, as a variety is cultivated in an unfavourable climate as in the case of red blood orange, the production of endogenous compounds that establishes the contact between the fruit and the calyx is affected and hence increase in the severity of citrus preharvest fruit drop.

5.3 Plant Nutrients Level from the Selected Areas

5.3.1 Nitrogen (N) Levels in Citrus Leaf and Fruit Peels

There was no significant difference from the leaf analysis among the selected areas but the fruit peels (dropped and un-dropped) had significant differences among them. From the leaf analysis, the nitrogen level at Ahafo Ano South (2.98 %) only falls within the optimum range of 2.5-3.0 % (Koo *et al.*, 1984). It is known that sufficient N concentration in the tree helps maximum vegetative growth, flowering and fruit yield when in balance with other nutrients (Obreza *et al*., 2011). But Better Crops, (1998) reported that proteins are not synthesized despite an abundance of available nitrogen when plants are deficient in K. However, at Bekwai Municipal and Adansi North, where the nitrogen levels fell below the optimum requirement, it implies that the required functions of nitrogen will not be performed sufficiently. Uchida, (2000) indicated that deficiency of N is known to cause early maturing in some crops which reduce yield and their quality. Allaby (2004) also indicated that, if all other nutrients are in abundance deficiency of a single nutrient will stop or influence growth. This implies that at Bekwai Municipal (2.29 %) and Adansi North (2.40 %), if all the other nutrients are sufficient and nitrogen is insufficient the required physiological roles will not be adequately done. Furthermore, Obreza *et al*., (2011) also reported that persistent N shortage result to defoliation, fruit drop and shoot death. Better

Crops (1998) revealed that when there is deficiency of nitrogen, it would result in translocation of N from older tissues to younger tissues.

5.3.2 Phosphorus (P) Levels in Citrus Leaf and Fruit Peels

Phosphorus levels from the citrus leaf were significantly different from each other among the selected areas. All the selected areas fell within the optimum range (0.12 – 0.16 %) of citrus leaf phosphorus requirement. Primary orthophosphate is mostly dominant in acid soils and its uptake is about ten (10) times as readily as the secondary orthophosphate form (Spectrum Analytic Inc., 2016). This means that if the pH falls within the acidic range, uptake of primary orthophosphate will be sufficient as required. The mean pH for all the selected areas; Bekwai Municipal (5.73), Ahafo Ano South (6.16) and Adansi North (5.78) fall within the acidic range. This may imply that if all the other essential nutrients are available in the right proportions the various physiological functions will adequately take place. However, Liebig's law of the minimum states that the rate of growth of a plant, the size to which the plant grows, and plant’s overall health depend on the amount of the scarcest of its essential nutrients that is available to the plant (Allaby, 2004). Thus, if all other nutrients are in abundance a deficiency of a single nutrient will stop or influence growth. This indicates that when there is deficiency of other nutrient(s) growth and yield will be affected. Phosphorus inversely correlated with potassium weakly to moderately in all the selected areas except late valencia variety at Adansi North (Tables 4.21). This negative interaction shows that the combined results are lower than the sum of the influences of the individual nutrients. Whilst at Adansi North and Ahafo Ano South, the correlation between phosphorus and zinc is strongly direct (0.75) and moderately direct respectively but they were not statistically significant. When there is proper balance of both phosphorus (P) and zinc (Zn) it results to normal growth and adequate yield. When there was adequate level of P and deficient Zn it revealed positive relationship at Adansi North (Tables 4.12) and Ahafo Ano South (Tables 4.10)**.**

5.3.3 Potassium (K) Levels in Citrus Leaf and Fruit Peels

The levels of potassium were significantly different among the areas from the leaf analysis. Though, there is significant difference among the selected areas they all fell far below the optimum requirement (1.2 – 1.7 %) of potassium. Thus about 50 % lower than the optimum requirement (1.2 %). This shows that the weaker the interactions, the lower the performance. Insufficient K leads to increase production losses and reduces drought or stress resistance as well as lowering the turgor of the citrus plants. Potassium plays an important role in the transport of nutrients and water throughout the plant in the xylem (Zekri and Obreza, 2015). When K supply is minimized, the translocation of nitrates, magnesium (Mg), amino acids, phosphates and calcium is inhibited. Plants deficient in K are not able to absorb water and are more subject to stress when water is in short supply. Inadequate potassium implies that Adenosine Triosphosphate is less available and the transport system (phloem and xylem transports) is broken down. This may mean that the more insufficient K is in a plant, the weaker the transport system (Adansi North <Bekwai Municipal< Ahafo Ano South). Moreover, inadequate supply of K affects the efficient operation of plants growth hormones (Better Crops, 1998). This inefficiency of phloem and xylem transport systems causes photosynthates to build up in the leaves and the rate of photosynthesis is also reduced. Deficiency in K implies that proteins are not synthesized even in abundance of available nitrogen (Better Crops, 1998). K deficiency shows that all the optimum performance required from potassium and zinc in citrus plants to reduce or delay abscission and disease infection (Kazokas, 1997; Spectrum Analytic Inc., 2016) will be low.

Phosphorus inversely reacted with insufficient potassium. This indicates that the optimum performance of these two nutrients will be lower than their individual performance. Better Crops (1998) reported that P-K interaction have synergistic effect on performance or yield. Potassium weakly associated with zinc inversely at Ahafo Ano South (-0.14) whiles at Bekwai Municipal (0.76) and Adansi North (0.72) there is strong relationship for late valencia. The basic function of potassium with zinc is expected to be lower than expected at Ahafo Ano South though adequate level of K increases utilization of micronutrients such as Cu, Zn and Mg. Potassium weakly reacted with zinc at Ahafo Ano South, Adansi North and Bekwai Municipal. This reveals that since there is weak interaction between K and Zn, the expected interactive functions will not be sufficiently done with respect to red blood oranges (Better crops, 1998).

5.3.4 Zinc (Zn) Levels in Citrus Leaf and Fruit Peels

With the leaf analysis, there were significant differences among the selected areas (Table 4.8). Though, there are significant differences among the areas but they are all far lower or deficient than the optimum requirement of zinc (25 – 100 mg/kg), according to Koo *et al.* (1984). The difference between the area with the highest level of zinc (6.69 mg/kg) and the minimum requirement of 25 mg/kg is over 70 % deficient or lower. Zinc availability is mostly dependent on pH and it is known to be higher in acidic soils. While deficiency is common on soils with neutral or alkaline pH that are sandy with low organic matter content (Nutrient Technologies, 2001). This implies that if there is adequate Zn available, it uptake should be higher. However, the pH of most of the farms were acidic and adequate level of plant phosphorus in almost all the selected areas or farms but there were low levels of available Zn, it implies that according to Nutrient Technologies (2001), zinc is ideally deficient. The problem is that most farmers usually do not apply fertilizer(s) and even those who apply do not know of zinc and its essence.

This may imply that the required role of zinc in the plant will be insufficiently felt. This is because Jeyakumar (2011) revealed that deficiency of micronutrients causes many more disorders than that of macronutrients. For instance, metabolism of plant hormones such as auxin (IAA) and tryptophan decreases in zinc deficient condition; as a result leaf growth stops (Pedler *et al*., 2000). When zinc is deficient, terminal growth areas (tip of shoot, old and growing leaves) are affected first. As the total available zinc is inadequate, plants normally suffer from physiological stress brought about by the dysfunction of several enzyme systems and other metabolic functions in which zinc plays part (Alloway, 2008). The deficient zinc correlated with phosphorus at Ahafo Ano South (0.41) directly moderate, had no relation at Bekwai Municipal but strong relationship at Adansi North (0.75) for leaf analysis from late valencia variety. Whiles for red blood variety, zinc correlates with P weakly at Ahafo Ano South (-0.22) inversely and no association at Bekwai Municipal (-0.06) and Adansi North (-0.08).

5.3.5 Comparison of Nutrient Values between the Varieties

There was no significant difference among all the nutrients from leaf and fruit peels (drop and un-dropped) between late valencia and red blood varieties. Differences are not mostly expected among varieties (Thomas Adjei-Gyapong, Personal Communication, April 23, 2016) of a crop in their nutrients uptake. There is no significant difference between the two varieties when their values were subjected to analysis of variance (Table 4.9). Duncan (2012) reported that though differences may not be seen among varieties in terms of their nutrients uptake, there can be cases where a variety can be an efficient converter of nutrients into dry matter. Also, some of the varieties are efficient when grown at lower levels of particular nutrients. It can be suggested that late valencia variety is efficient on lower levels of nutrients than red blood oranges in tropical regions.

5.3.6 Leaf-Drop fruit Peel Zinc (Zn) and Nitrogen (N) Relationships (r) from the Selected Areas

Nitrogen and zinc in citrus trees are involved in the bio-synthesis of plant hormone, auxin (indole acetic acid) (NPCS Board of Consultant and Engineers, 2009) which reduces fruit abscission or drop. The synthesis of this auxin reduces or delays fruits from dropping or abscising (Albrigo, 2014) but NPCS Board of Consultant and Engineers, (2009) have indicated that deficiency of zinc is next to nitrogen deficiency. So relationship (r) between nitogen and zinc from leaf and dropped fruit peels were considered from the selected areas. It was observed that the perfect and weaker relationship between leaf and dropped fruit peel for zinc (0.87) and nitrogen (0.20) resulted in the higher mean fruit drop (108) for late valencia at Ahafo Ano South. The direct association for zinc (0.63) and inverse relationship (r) for nitrogen resulted in the highest fruit drop of red blood variety (228). With the lowest mean fruit drop at Adansi North, the late valencia had direct relationship for zinc (0.49) and inverse relationship for nitrogen (-0.39). But red blood variety at Adansi North had an inverse relationship for zinc (-0.69) and weak association for nitrogen (0.25), (Table 4.13). Though, there was no significant difference between late valencia and red blood for leaf total nitrogen and zinc levels (Table 4.9). In addition, zinc levels for both varieties fall within insufficient range (< 25 mg/kg) (Koo *et al.*, 1984) whilst nitrogen levels are sufficient for the varieties. The late valencia seems to be efficient with the nutrients utilization as compared to red blood variety (Duncan, 2012) since red blood oranges dropped twice as much as late valencia.

5.4 Pathogens or Lesions Identified

5.4.1 Pathogens Identified from Cultured Citrus Black Spot lesions

The following were the pathogens found on the dropped fruit black lesions (Table 4.14) *Phyllosticta citricarpa, Aspergillus niger, Aspergillus flavus* and *Collectotrichum gloeosporoides* from both late valencia and red blood oranges or varieties from all the selected areas. Tafinta *et al*., (2013), reported that *Apergillus niger, Aspergillus flavus, Apergillus fumigatus and Rhizopus stolonifera* are postharvest pathogens that are associated with spoilage of sweet orange with frequencies of occurrence as 17 %, 25 %, 22 % and 36 % respectively. From this work, the percentage distribution for *Apergillus niger* and *Aspergillus flavus* were 30 % and 15 % for late valencia and 20 % and 26 % for red blood oranges respectively. These two pathogens are not yet known and reported to be sweet orange (*citrus sinensis*) pre-harvest pathogens. Onuorah *et al*., (2015) reported that sweet oranges are prone to spoilage by filamentous fungi such as *Apergillus niger* and *Aspergillus flavus* as a result of their high levels of sugars and low pH values and added that Aspergillus niger cause the highest degree of spoilage.

Anthracnose is caused by a fungal pathogen *Colletotrichum gloeosporioides* (Penz.) which grows and sporulates in deadwood on the trees, with water transmitting spores to the immature fruit surface where the fungus forms infection structures known as appressoria. These appressoria remain latent and do not cause decay prior to harvest (Kader, 2002). Lieberman, (1983) indicated that nitrogenous compounds that are released to the surface of the fruit delay the formation of infection structure such as appressoia. Considering the three forms of *Collectotrichum* spp. that are recognized on citrus: fastgrowing gray (*C. gloeosporoides*) form, the slow-growing orange form and the key lime anthracnose.

Pathogenicity tests has confirmed that slow-growing orange isolates infects sweet orange flowers only, the isolates of key lime anthracnose infects sweet orange flowers and key lime foliage. The pathogen identified as key lime anthracnose and slow growing orange is *Collectotrichum acutatum* (Brown *et al*., 1996). This shows that Collectrichum spp do not infect mature citrus fruits not detached from the parent plant though appressoria may grow and remain latent till fruit(s) is detached from the tree. Kuramae-Izioka *et al*., (1997) from Brazil reported on the genetic characterization of *Colletotrichum* isolates which suggest that postbloom fruit drop (fruit set drop) can be caused by both *C. acutatum* and *C. gloeosporioides*. However, Aiello *et al*., (2014) reported on the pre-harvest infection of *Colletotrichum* spp. causing anthracnose disease on citrus but Brown *et al.*, (1996) have indicated that the pathogen, *Colletotrichum gloeosporoides* is a saprophyte and a postharvest pathogen. Whilst Kotzé, (1981) and Kader, (2002) have shown that appressoria can remain latent till fruit(s) is detached from the tree. The pathogenicity test done by Aiello *et al*., (2014) should have been done on fruits that are not detached from the parent plant to confirm pre-harvest infection of *Colletotrichum gloeosporoides*. This is due to the fact that *Collectotrichum gloesporoides* has been reported to remain latent at the appressoria stage on the fruits till it is detached from the parent plant before it can infect citrus fruit. So for confirmation of *Collectotrichum gloesporoides* pre-harvest infection, *in vivo* could have helped. The fruits that were collected, isolated and identified were picked from the ground which indicates that a saprophyte is likely to grow on the fruits to facilitate decay. Kotze (2000) reported that *Collectotrichum* spp. is an endophytic fungus and Peres *et al.*, (2007) and IPPC, (2014) indicated that they often can overgrow some slow-growing pathogens such as *Phyllosticta citricarpa.*

Phyllosticta citricarpa is a pre-harvest pathogen which is the causal pathogen of citrus black spot. The critical stage for infection starts from fruit set stage and remains susceptible to infection until 4–6 months as symptoms on fruit is not known to appear immediately after fruit set (Baldassari *et al*., 2008 ; Truter *et al*., 2004). According to the farmers, the black spot symptoms are seen when the fruits have reached the green mature size which opposes Kotzé (1981), report that symptom mostly occurs following the period fruit has changed from green to orange colour. Kotzé, (2000) reported that symptom development on mature fruit is enhanced by rising temperature, high light intensity, drought and poor tree vigour. Several symptoms such as; hard spot, freckle spot, false melanoses and virulent spot appear on fruit, depending on the temperature and fruit maturity. These symptoms were observed in all the farms visited in all the selected areas or farms in Ashanti Region.

The presence of the symptoms, according to farmers, induces or forces the fruit to develop the orange colour or ripe immaturely. Thus when the fruits total soluble solids and acid ratio have not appropriately balanced for consumption or harvesting.

Sweet orange is a non-climateric fruit, internal ripening has different physiological process from the external ripening (Iglesias *et al.*, 2007). So according to farmers as the peel ripens, the pulp would not have been edible, and then it drops before the usual harvesting time. Citrus pre-harvest fruit drop that increases crop losses has been linked with citrus black spot and have been assessed to be 71% in Ghana (Brentu *et al*., 2012) and up to 80% in untreated groves in Australia and South America (Calavan, 1960).

5.4.2 Leaf Nutrients level and Rate of Fruit Drop with Black Spot Lesions from the Selected Areas

With late valencia variety, Adansi North had the highest level of zinc (7.86 mg/kg), lowest level of potassium (0.41 %) and the highest incidence of citrus black spot lesions

(89.64 %). For red blood variety, Bekwai Municipal had the lowest level of potassium (0.41 %) concentration, the highest level of zinc (6.02 mg/kg) and the highest level of citrus black spot lesions (92.18 %) at Bekwai Municipal (Table 4.15). Zinc and potassium are about 65 % to 75 % deficient from the minimum optimum requirement. Any citrus plant that is nutritionally stressed increases the chances of citrus black spot infection (http://america.pink/citrus-black-spot_1003068.html). Spann and Schumann, (2013) indicated that mineral imbalances or insufficiency lower resistance of the plant to diseases by creating a more favourable environment for pathogens (such as fungus, bacteria, nematodes and virus) to affect the formation of mechanical barriers in plant tissue. It can be observed that as potassium and zinc become more deficient the higher the rate of incidence of citrus black spot lesions at Bekwai Municipal and Adansi North. Shortages of key nutrients reduce the amount of the plants natural antifungal compounds at the site of infection on the fruits. In addition, Spann and Schumann, (2013) reported that as pathogen infects the deficient plant; it immobilizes nutrients in infected tissues and interferes with translocation or utilization of nutrients and induces nutrient deficiencies or toxicities. This implies that the pathogen, *Phyllosticta citricarpa* was able to alter the plant's physiology, with regard to mineral nutrient assimilation, translocation and utilization.

Moreover, high Zn nutritional level or fertilization controls both fungus and virus infections (Spann and Schumann, 2013). Also, when there is optimum level of potassium, it alters the compatibility relationship of the host-parasite environment within the plant. But when K is deficient, it lowers crops resistance to disease infections and plant becomes susceptible to fungus since its natural defenses are broken. Shortage of K reduces the amount of the plants natural antifungal compounds at the site of infection. The infection causes increase production of fungus inhibiting phenolic compounds and flavonoids, both at the site of infection and in other parts of the plant (Spectrum Analytic Inc., 2016). This reveals that the more zinc and potassium are deficient, the lower the synthesis of the anti-fungal compounds. Therefore, the more insufficient potassium and zinc levels, the higher the incidence of fungal disease, citrus black spot.

5.5 Possible Causes of Pre-harvest Fruit Drop in the Major Citrus Growing Areas

Albrigo (2014) proposed fruit drop process and stated that it is induced by stress. According to him, it starts with *stress* then *ethylene* production followed by *cell wall breakdown at fruit abscission zone* and finally *abscission of fruit* (fruit drop). He defined stress to be; carbohydrate deficiency, water stress, fruit peel senescence, nutrient imbalance or deficiency and pathological infection. He revealed that the stress that induces citrus pre-harvest fruit drop in Florida is caused by citrus greening (HLB) plus additional environmental stress. In addition, two main causes of citrus pre-harvest fruit drop according to NPCS Board of Consultants and Engineers, (2009) are physiological and pathological factors but all the two fall under stress. Physiological cause was defined to be deficiency or excess of certain essential nutrients, faulty cultural practices and imbalances of growth regulators such as auxin and cytokinin.

It can be explained that, pre-harvest fruit drop in the major citrus growing areas is caused by pathological infection (*Phyllosticta citricarpa*), nutritional stress or deficiency and other unknown stresses. This is because in all the selected areas, nitrogen, potassium and zinc nutrients necessary for anti-fungal compounds and auxin productions or synthesis were all insufficient or imbalance. But the process that leads to fruit drop disagrees partially with the proposal made by Albrigo (2014). This is because Iglesias *et al*. (2007) reported on citrus fruit physiology and indicated that there is no evidence that any specific hormone controls the whole process of ripening. Thus, the second stage of the process, ethylene production is not responsible for ripening in citrus. Though, ripening in non-climacteric fruit follows ethylene-dependent event (Barry and Giovannonni, 2007), there is absence of rise in respiration and autocatalytic ethylene production (McManus, 2012). Giovannoni (2004) reported that the biochemical processes involved in ripening phenomena varies by species. They vary through modification of sugars, acids; volatile profiles that affect nutritional quality, flavor and aroma and enhancing susceptibility to opportunistic pathogens associated with loss of cell wall integrity. In addition, the process of external citrus fruit ripening is mostly dependent upon the conversion of chloro- to chromoplasts and involves the progressive loss of chlorophylls and the gain of carotenoids, thus changing peel color from green to orange (Iglesias *et al.*, 2007). Moreover, according to some farmers, citrus black spot forces sweet orange to ripen prematurely. This shows that the black spot symptom causes the fruit peel to break its cell wall and the plant nutrition is utilized by fungus to produce inhibiting phenolic compounds and flavonoids (Spectrum Analytic Inc., 2016) which facilitate the premature ripening and abscission or dropping of fruits.

Therefore, *Phyllosticta citricarpa* (citrus black spot), deficiency of N, K and Zn (nutritional) and other factors are the stresses that induced or forced ripening that was followed by breakdown of cell wall at fruit abscission zone and finally abscission of fruit (fruit drop).

Citrus Black Spot + Nutritional + Additional factors → forced ripening → Breakdown of cell walls in fruit abscission zone → Abscission of fruit (fruit drop)

Stress

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This research was conducted to

- assess farmers' perception on the causes of citrus pre-harvest drop and their management practices.

- determine the severity of citrus pre-harvest fruit drop in the major citrus growing areas.
- determine the soil and plant nutritional status of nutrients associated with citrus pre-harvest fruit drop.
- identify pathogens associated with citrus pre-harvest fruit drop

6.1 Conclusion

- All the citrus farmers were aware of citrus fruit pre-harvest drop. Almost all the farmers had perception that fruit flies and citrus black spot disease caused citrus pre-harvest fruit drop. They are of the view that scent from flowers and scent or orange colour of mature fruits (40 %) attracts this insect pest to the citrus farms to pierce and lay eggs into the fruits. Though, one-third did not control the fruit flies but 28.3 % combine bait application and pheromone trap in their orchards and they claimed the combination is effective. Moreover, the farmers indicated that black spot symptoms are seen on matured green fruits then it forces the fruits to ripe, which is called '*kyembedie*' in the local language. However, about one-half indicated that nothing was done to control the disease even as 21.6 % control citrus black spots by mixing both fungicides and insecticides to spray as their control measure to reduce citrus black spot disease.

- Red blood variety drops more than late valencia in all the major citrus growing areas. The highest mean for red blood variety is 228 whilst that of late valencia is 109. Severity of pre-harvest fruit drop might have been influenced or facilitated by climate, variety cultivated and cultural practices such as weed control, pruning and fertilizer application. Again, unfavourable climate might have compelled red blood orange to drop more than late Valencia in Ashanti Region.

- The leaf nutritional status for phosphorus at Ahafo Ano South (0.17 %) was not significantly different from Adansi North (0.15 %) but over Bekwai Municipal (0.14 %). For K; Ahafo Ano South (0.60 %) was significant over Bekwai Municipal (0.45 %) and Adansi North (AN) (0.42 %). With zinc, Bekwai Municipal (6.52 mg/kg) and Adansi North (6.69 mg/kg) were significant over the Ahafo Ano South (4.88) and for nitrogen there was no significant difference. It was observed that the perfect and weaker the relationship (r) between leaf and dropped fruit peels for zinc (0.87) and nitrogen (0.20) respectively, the higher the mean fruit drop (108) for late valencia. With red blood variety, the moderate the direct association for zinc (0.63) and the inverse the relationship for nitrogen (0.37) resulted to the higher mean fruit drop (228) at Ahafo Ano South. The more insufficient potassium and zinc levels, the higher the incidence of citrus black spot lesions for late valencia (89.64 %) at Adansi North and red blood (92.18 %) varieties at Bekwai Municipal.
- The pathogens that were found on the dropped fruit black spot lesions are *Phyllosticta citricarpa, Aspergillus niger, Aspergillus flavus* and *Collectotrichum gloeosporoides* from both late valencia and red blood varieties from all the selected areas. It is *Phyllosticta citricarpa* that is known to cause citrus preharvest infection and lead to pre-harvest fruit drop.

6.2 Recommendations

- Farmers should be trained on appropriate farm practices such as fertilizer application, pruning and selection of plant materials to enable them to reduce the incidence of fruit drop.
- Farmers should be informed that all dropped citrus black spot infected fruits should be collected and bagged in a transparent polyethlyne and expose it to high temperature (sunlight) to control the multiplication of the pathogen before new flowering season
- Further research should be conducted to control pre-harvest fruit drop by applying foliar fertilizer containing (N, P, K and Z n) essential nutrients associated with citrus pre-harvest fruit drop.

REFERENCES

Adalton, R., Daniela, A.O. P., Miguel, F. S. F. and Mário, E. S., (2004). Fruit fly (Diptera: Tephritoidea) infestation in citrus in the State of São Paulo, Brazil, Neotropical Entomology, vol.33 no.1 http://dx.doi.org/10.1590/S1519

Aiello, D., Carrieri, R., Guarnaccia, V. and Vitale, A., (2014). Characterization and Pathogenicity of *Colletotrichum gloeosporioides* and C. karstii Causing Preharvest Disease on Citrus sinensis in Italy, *Journal Phytopathology*, 163 (2015) 168–177

Albrigo, L.G., (2014). Pre-harvest fruit drop –HLB relationships 2013-14 Season, Citrus Research and Education Center, University of Florida, IFAS extension

Albrigo, L. G., (2013). Fruit drop – Historical and current perspective, Citrus Research and Education Center, University of Florida, IFAS extension,

Alloway, B.J., (2008). Zinc in Soils and Crop nutrition. 2nd edition, Published by IZA and IFA, Brussels, Belgium and Paris, France

Allaby, M., (2004). "Liebigs law of the minimum." A Dictionary of Ecology, *Encyclopedia.com* retrieved on 23/03/2016 from http://www.encyclopedia.com.

Appiah, E. F., Afreh-Nuamah, K. and Obeng-Ofori, D., (2009). Abundance and distribution of the Mediterranean fruit fly *Ceratitis capitata* (Diptera:Tephritidae), in Late Valencia citrus orchards in Ghana, International Journal of Tropical Insect Science, vol 29(01):11 –16

Ashraf, M.Y., Yaqub, M., Akhtar, J., Khan M. A. and Ebert, G., (2012). Control of Excessive Fruit Drop and Improvement in Yield and Juice Quality of Kinnow (*Citrus deliciosa* x *Citrus nobilis*) Through Nutrient Management, Pak. J. Bot., 44: 259-265

Atta, S., Zhou, C. Y., Zhou, Y., Cao, M. J. and Wang, X. F., (2012). Distribution and Research Advances of *Citrus tristeza* vírus, *Journal of Integrative Agriculture*, vol. 11, No. 3, pp. 346-358.

Azuma, R., Kurata, H., Adachi, M. and Shimokawa, K., (1999). Degreening of *Citru unshiu* fruits via ethylene induced soluble chlorophyllase. J. Jap. Soc. Hort. Sci. 68:558-562.

Baayen, R. P., Bonants, P. J. M., Verkley, G., Carroll, G. C. and Van der Aa, H.A., (2002). Nonpathogenic isolates of the citrus blackspot fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology* 92: 464-477.

Badii K. B., Billah M. K., Afreh-Nuamah K. and Obeng-Ofori D., (2012). Farmers' Knowledge and Perceptions of Fruit Fly Pests and Their Management in Northern Ghana, *Greener Journal of Agricultural Sciences*, ISSN: 2276-7770 Vol. 2 (8), pp. 412-423.

Baghurst, K., (2003). The Health Benefits of Citrus Fruits **-** CSIRO Health Sciences and Nutrition**,** Horticultural Australia Ltd, ISBN 0 7341 0694 7

Baldassari, R. B., Wickert, E. and De Goes, A. (2008). Pathogenicity, colony morphology and diversity of isolates of *Guignardia citricarpa* and *G. mangiferae* isolated from *Citrus* spp. *European Journal of Plant Pathology* 120:103-110.

Better Crops, (1998). Vol. 82 (No. 3), *https://www.ipni.net/ppiweb/b**crops**.nsf/$webindex/.../$file/98-3p04.pdf*

Ben-Arie, R., Mignani, I., Greve, C., Huysamer, M. and Labavitch, J. M., (1995). Regulation of the ripening of tomato pericarp discs by GA_3 and divalent cations. Physiology of Plant 93:99-107.

Bernardi, J., Licciardello, C., Russo, M.P., Chiusano, M. L., Carletti, G., (2010). Use of a custom array to study differentially expressed genes during blood orange (*Citrus sinensis* L. Osbeck) ripening, *Journal of Plant Physiology*, vol. 167, pp. 301–310

Bonants, P. J. M., Carroll, G. C., de Weerdt, M., van Brouwershaven, I. R. and Baayen,

R. P. (2003). Development and validation of a fast PCR-based detection method for pathogenic isolates of the citrus black spot fungus, *Guignardia citricarpa*. *European Journal of Plant Pathology* **109**: 503-513.

Borochov-Neori, H., Judeinstein, S., Harari, M., Bar-Ya'akov, I. and Patil, B. S., (2011). Climate Effects on Anthocyanin Accumulation and Composition in the

Pomegranate (*Punica granatum* L.) Fruit Arils, *J. Agric. Food Chem.*, 59:10, pp 5325–5334 DOI: 10.1021/jf2003688, http://pubs.acs.org/doi/abs/10.1021/jf2003688

Brentu, F. C., Cornelius, E. W., Lawson, L. E. V. and Oduro, K. A., (2013). First Report of *Pseudocercospora angolensis* Causing Fruit and Leaf Spot of Citrus in Ghana, *The American Phytopathological Society,* Volume 97, Number 12, Page 1661, http://dx.doi.org/10.1094/PDIS-06-13-0615-PDN

Brentu, F., Oduro, K., Offei, S., Odamtten, G. and Vicent, A., (2012). Crop loss, Aetiology and Epidemiology of citrus black spot in Ghana, *European J. Plant Patholo*gy, 133:657–670

Brodrick, H. T. (1969). Physiological studies with *Guignardia citricarpa* Kiely, DSc. Thesis, University of Pretoria, Pretoria

Brown, A. E., Sreenivasaprasad, S., and Timmer, L. W., (1996). Molecular Characterization of slow-ggrowing orange and Key lime antracnose strains of *Colletotrichum* from citrus as *Colletotrichum acutatum.*

Butelli, E., Licciardello, C., Zhang,Y., Liu, J., Mackay, S. and Bailey, P., (2012). Retrotransposons control fruit-specific, cold-dependent accumulation of anthocyanins in blood oranges. Plant Cell 10.1105/tpc.111.095232, retrieved from http://www.plantcell.org/

Calavan, E. C., (1960). Black spot of citrus, California Citrograph 46:4, 18, 22–24.

Cha, J. Y., Cho, Y. S., Kim, I., and Anno, T., (2001). Effect of hesperetin, a citrus flavonoid on the liver triacylglycerol content and phosphatidate phosphohydrolase activity in orotic acid-fed rats, *Plant Foods for Human Nutrition*, 56, 349–358 **Chung, K. R. and Timmer, L. W. (2009).** Citrus diseases exotic to Florida: *Phaeoramularia* fruit and leaf spot (PFLS). Fact Sheet PP-234, Gainesville, USA: University of Florida, retrieved from: http://edis.ifas.ufl.edu/pp155. 28/10/2015.

Crowell, P. L., (1999). Prevention and therapy of cancer by dietary monoterpenes, *Journal of Nutrition*, www.ncbi.nlm.nih.gov/pubmed/10082788, *9/09/2015*

Davies, F. S. and Albrigo, L. G., (2006). Citrus, Crop Production Science in Horticulture 2, University of Florida, CAB Internatinal, ISBN 0-85198-867-9

Davies, F. S. and Albrigo, L. G., (1998). Citrus, CAB International, Wallingford, UK

Davies, F. S., (2002). Fruit Drop Problems of Citrus, CAB International, Wallingford, UK

Department of Agriculture and Food, (2016). https://www.agric.wa.gov.au/fruit/mediterranean-fruit-fly, date retrieved - 18/02/16

Duncan, R., (2012). Crops an Enhancers of nutrient use, Acadmic Press

Ehsani, R., (2007). In-situ Measurement of the Actual Detachment Force of Oranges Harvested by a Canopy Shaker Harvesting Machine, Abstracts for the 2007 Joint Annual Meeting of the Florida State Horticulture Society.

Elangovan, V., Sekar, N. and Govindasamy, S. (1994). Chemopreventive potential of dietary bio-flavonoids against 20-methylcholanthreneinduced tumorigenesis, *Cancer Letters*, 87, 107–113

Etebu, E. and Nwauzoma, A. B., (2014). A Review on Sweet Orange (*Citrus Sinensis* L Osbeck): Health, Diseases and Management, *American Journal of Research Communication*, www.usa-journals.com date accessed 8/07/15

EMQAP-MOFA (Export Marketing and Quality Awareness Project), **(2013).** Fruit fly Management in Ghana, Pp 28-29, ISBN: 978-9988-8562-4-3

Fageria, N. H, and Stone, L. F., (2008). Micronutrient Deficiency Problems in South America, Chapter 10, In Alloway,B.J. (ed.) *Micronutrient Deficiencies in Global Crop Production*, Springer, Dordrecht, pp 245-266.

FAO (Food and Agriculture Organization of the United Nations), (2009). FAO Statistics, http://faostat.fao.org/default.aspx;

FAOSTAT (Food and Agriculture Organization of the United Nations), (2013). FAO Statistics, http://faostat.fao.org/default.aspx;

(FAO) Food and Agriculture Organisation, (1998). Food, Nutrition and Agriculture, 12th Session of the Intergovernmental Group on Citrus Fruit, Valencia, Spain

Ferrandiz, C., Liljegren, S.J. and Yanofsky, M.F., (2000). Negative regulation of the shatterproof genes by fruit fall during Arabidopsis fruit development, Science 289, 436– 438

Fertilizing and Pruning, (2016). http://www.engalls.com.au/Citrus/CitrusCare/FertilisingandPruning/tabid/67/Default.aspx

Fujii, H., Shimada, T., Sugiyama, A., Nishikawa, F., Endo, T. and Nakano, M., (2007). Profiling ethylene-responsive genes in mature mandarin fruit using a citrus 22K oligoarray, Plant Sci.173:340-348

Giovannoni, J.J. (2004). Genetic Regulation of Fruit Development and Ripening, American Society of Plant Biologists-The Plant Cell, Vol. 16, S170–S180, retrieved from www.plantcell.org

Goldschmidt, E. E., (1988). Regulatory aspects of chlorochromoplast interconvensions in senescing Citrus fruit peel, Isr. J. Bot. 47:123-130.

Goudeau, D., Uratsu, S. L., Inoue, K., daSilva, F. G., Leslie, A., Cook, D., (2008). Tuning the orchestra: Selective gene regulation and orange fruit quality, *Plant Science*, 174, pp. 310– 320

Gross, J., (1987). Pigments in Fruits, Academic Press, London

Guis, M., Botondi, R., Ben-Amor, M., Ayub, R., Bouzayen, M. and Pech, J., (1997). Ripening-associated biochemical traits of Cantaloupe Charentais melons expressing an antisense ACC oxidase transgene, J. Am. Soc. Hort. Sci. 122:748751.

Guo, W.W. and Deng, X. X., (2001). Wide somatic hybrids of *Citrus* with its related genera and their potential in genetic improvement, *Euphytica* 118, 175-183

Heo, H. Y., Lee, S. J., Kwon, C. H. and Kin, S. W., (1994). Anticlastogenic effects of galangin against bleomycin induced chromosomal aberrations in mouse spleen lymphocytes, Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis, 311, 225–229

http://californiarangeland.ucdavis.edu/Mediterranean-climate

http://www.ecoca.ro/meteo/tutorial/Climate/Older/Climate_Zones.html

Iglesias, D. J., Tadeo, F. R., Legaz, F., Primo-Millo E. and Talon, M., (2001). In vivo sucrose stimulation of colour change in citrus fruit epicarps: interactions between nutritional and hormonal signals. Physiol. Plant. 112:244-250.

Iglesias, D. J., Cercós, M., Colmenero-Flores, J. M. and Naranjo, M. A., (2007). Physiology of citrus fruiting, Brazil Journal of Plant Physiology, 19(4):333-362.

International Plant Protection Convention (IPPC), (2014). International Standards for Phyto- sanitary Measures (ISPM) 27 Diagnostic Protocols (DP 5): *Phyllosticta citricarpa* (McAlpine) Aa on fruit, Rome, FAO, www.fao.org/3/a- ml513e.pdf, *12/11/201*

Jeyakumar, P., (2011). Physiological Disorders in Fruit Crops, Horticultural College and Research Institute, Pp 1-8

Kader, A. A., (2002). Postharvest Technology of Horticultural Crops, Technology and Engineering, https://books.google.com.gh/books?isbn=187990651, accessed on 6/04/16

Kahl, H., (2004). Role and importance of nitrogen in your soil, https://organicnz.org.nz/node/153 date retrieved 7/03/2016

Karim, M. F. and Neven, M.N.N., (2012). Effective Reduction of Postset and Preharvest Abscissions and Increasing the Yield of "Washington" Navel Orange Fruits by 1- MCP, GA3 and NAA., Journal of Applied Sciences Research, 8(10): 5132-5141, ISSN 1819-544X

Kazokas, W., (1997). Activities of cell wall hydro lases and cellulase gene expression in Valencia orange and Tahiti lime calyx abscission zones during intennittent ethylene treatments, M. Sc. Thesis, University of Florida.

Kellerman, C.R. and Kotze, J.M. (1977). The black spot disease of citrus and its control in South Africa. *Proceedings of the International Society of Citriculture* 3: 992-996.

Kelly, J. and Wright, G., (2010). Pruning Citrus, University of Arizona, College of Agriculture and Life Sciences, cals.arizona.edu/pubs/crops/az1455.pdf **Keys, A., (1995).** Mediterranean diet and public health: Personal reflections, *American Journal of Nutrition* 129 (3): 775S–778S, PMID 10082788.

Khalid, M. S., Amin, M., Hafeez, O., Haider, F. and Umar, M., (2013). Role of Pruning in Citrus, Timing and its Impact on Tree and Fruit, https://agrinfobank.wordpress.com retrieved on 18/02/16

Kiely, T. B., (1948). Preliminary studies of *Guignardia citricarpa*, n.sp.: the ascigerous stage of *Phoma citricarpa* McAlp. and its relation to black spot of citrus. *Proceedings of the Linnean Society of New South Wales* **73**: 249-291.

Kimball, D. A., (1999). Citrus processing: a complete guide *(2nd ed.). New York: Springer: 450.* ISBN 0-8342-1258-7 Knowledge Master, (2016), http://www.extento.hawaii.edu, date retrieved -19/05/16

Kotzé, J. M., (2000). Black spot. *In* L.W. Timmer, S.M. Garnsey and J. H. Graham, eds. *Compendium of Citrus Diseases*, 2nd edn, pp. 23–25. Saint Paul, MN, USA, APS Press. 128 pp.

Kotzé, J. M., (1996). History and epidemiology of Citrus Black Spot in South Africa, In Proceedings of the International Society of Citriculture, pp. 1296-1299.

Kotzé, J. M., (1981). Epidemiology and control of citrus black spot in South Africa, *Plant Disease* **65**: 945-950.

Kuate, J., Foure, E., Foko, J., Ducelier, D. and Tchio, F., (2002). Citrus phaeoramulariosis due to *Phaeoramularia angolensis* in Cameroon: parasitic expression at different altitudes. *Fruits* 57:207-218.

Lieberman, M., (1983). Post-Harvest Physiology and Crop Preservation, NATO advanced study institutes series, Series A,, Life sciences 46, retrieved on 6/04/16, from https://books.google.com.gh/books?isbn=1475700946

Lo Piero, A. R., Consoli, A., Puglisi, I. and Orestano, G., (2005). Anthocyaninless Cultivars of Sweet Orange lack to Express the UDP-Glucose Flavanoid 3-OGlucosyl Transferase, *J. Plant Biochemistry and Biotechnology*, vol. 14, pp 9-14

McManus, T. M., (2012). Annual Plant Review, The Plant Hormone Ethylene, retrieved from *https://books.google.com.gh/books?isbn=111822311X*

Meredith, F. I. and Young, R. H., (1969). Effect of Temperature on Pigment development in red-blush Grape fruit and Ruby blood orange, Proceedings from fruit International Citrus Symposium, Vol. 1, pp 271–276

Miles, A. K., Willingham, S. L. and Cooke, A. W. (2004). Field evaluation of the strobilurins and a plant activator for the control of citrus black spot, *Australasian Plant Pathology* **33**: 371-378.

Morton, J., (1987). Orange. p. 134–142. In: Fruits of warm climates. Julia F. Morton, Miami, FL. https://www.hort.purdue.edu/newcrop/morton/orange.html

Mousavi, S. R., (2011). Zinc in Crop Production and Interaction with Phosphorus, Australian Journal of Basic and Applied Sciences, 5(9): 1503-1509, ISSN 1991-8178

Nartvaranant, P., (2012). Physiological Changes in Pre-Harvest Dropped Fruits in the Pummelo Cultivars '*Thong Dee*' and '*Khao Nam Phueng*', Songklanakarin J. Sci. Technology, http://www.sjst.psu.ac.th

Nault, B. A., Hsu, C. L. and Hoepting, C. A., (2011). Do Tank Mixes of Insecticides and Fungicides Affect Thrips Control? Department of Entomology, Cornell University

NPCS Board of Consultant and Engineers, (2009). Handbook on Citrus fruits Cultivation and Oil extraction, Pp 56-57, http://book.google.com.gh/book date 12/02/2016

Nutrient Technologies – Essential Plant Nutrients and their functions, (2001). Western Fertilizer Handbook, 2nd Horticulture Ed., California Fertilizer Association, date accessed: 23/09/2015 http://www.techflo.com/TechBulletins/NutrientFncts2.PDF

Obreza, T. A., Zekri, M. and Futch, S. H., (2011). General Soil Fertility and Citrus Tree Nutrition in *Nutrition of Florida Citrus Trees*, website: http://edis.ifas.ufl.edu, date accessed: 9/11/2015

Onuorah S., Ifeanyi, O., Ugochukwu, O., (2015). Filamentous Fungi Associated with the Spoilage of Post-Harvest Sweet Orange Fruits (Citrus Sinensis) Sold in Awka Major Markets, Nigeria, Bioengineering and Bioscience 3(3): 44-49, http://www.hrpub.org DOI: 10.13189/bb.2015.030303

Osman, K. T., (2012). Soils: Principles, Properties and Management, Pp 113, https://books.google.com.gh/books?isbn=9400756631

Owusu-Donkor, Y., (2011). Heavy Metal Contents of Soil and Citrus Grown in Selected Districts of Ashanti Region, Master of Science in Soil Science Thesis, Kwame Nkrumah University of Science and Technology

Paul, I., Jaarsveld, A. S. V., Korsten, L. and Hattingh, V., (2005). The potential global geographical distribution of citrus black spot caused by *Guignardia citricarpa* (Kiely): likelihood of disease establishment in the European Union. *Crop Protection* **24**: 297 308.

Pazoti, M. A., Garcia, R. E., Pessoa, J. D. C. and Bruno, O. M., (2005). Comparison of shape analysis methods for *Guinardia citricarpa* ascospore characterization, *Electronic Journal of Biotechnology*, http://www.ejbiotechnology.info/content/vol8/issue3/full/1/

Peres, N. A., Harakava, R., Caroll, G. C., Adaskaveg, J. E. and Timmer, L.W. (2007). Comparison of molecular procedures for detection and identification of *Guignardia citricarpa* and *G. mangiferae*. *Plant Disease*, 91: 525–531.

Pedler, J.F., Parker, D.R. and Crowley, D.E., (2000). Zinc Deficiency-induced phyto-siderophore release by the Triticaceae is not consistently expressed in solution culture. Planta, 211: 120-126.

Piccinelli, A. L., Mesa, M. G., Armenteros, D. M., Alfonso, M. A. and Rastrelli, L., (2008). HPLC- PDA-MS and NMR Characterization of C-Glycosyl Flavones in a Hydroalcoholic Extract of *Citrus aurantifolia* Leaves with Antiplatelet Activity, J. Agric. Food Chem. 56: 157.

Quaggio J. A., Mattos Jr D., Cantarella H., Stuchi E. S. and Sempionato O. R., (2004). Sweet orange trees grafted on selected rootstocks fertilized with nitrogen. phosphorus and potassium. *Pesquisa Agropecuária Brasileira* 39, 55-60.

Roeder, A.H., Ferrandiz, C. and Yanofsky, M.F., (2003). The role of the replumless homeodomain protein in patterning the Arabidopsis fruit. Curr. Biol. 13, 1630–1635.

Sbaraglia, M., (2016). Guide to Interpretation of Soil Analysis, Agronomical aspects of software Pedon – Ghana

Sajid, M., Rab, A., Ali, N., Arif, M., Ferguson L. and Ahmed, M., (2010). Effect of Foliar Application of Zn and B on fruit production and physiological disorders in sweet orange cv. Blood orange. Sarhad J. Agric. 26(3): 355-360

Samson, J.A., (1986). Citrus Tropical Fruits, *Longman Scientific and Technical Essex*, New York, Pp 73-138.

Savita, G. S. V. and Nagpal, A., (2012). Citrus diseases caused by *Phytophthora* species *Phytophthora* spp. GERF Bulletin of Bioscience, Pp 3(1):18-27

Schubert, T., Sutton, B. and Jeyaprakash, A., (2010). Citrus black spot (*Guignardia citricarpa*) discovered in Florida, Florida Department of Agriculture and Consumer Services, Pest Alert DACS-P-01723

Schutte, G. C, Mansfield, R.I., Smith, H. and Beeton, K. V., (2003). Application of Azoxystrobin for control of benomyl-resistant *Guignardia citricarpa* on Valencia oranges in South Africa. *Plant Disease* **87**: 784-788.

Smith, I. M., McNamara, D. G., Scott, P. R. and Holderness, M., (1997).

Quarantine pests for Europe. CAB International, Wallingford.

Smith, J. H. (1996). A study of the effect of various disease control programs on spore releases of the citrus black spot pathogen *Guignardia citricarpa* Kiely, In Proceedings of the International Society of Citriculture, pp. 351-352

Spectrum Analytic Inc., (2016). http://www.spectrumanalytic.com/support/library/ff/P_Basics.htm

Spiegel-Roy, P., Goldschmidt, E. E., (1996). Biology of Citrus, Cambridge University Press, Cambridge.

Soltész, M., (2002). Alma In: Nyeki, J. – Soltesz, M. – Szabo, Z. (ed.) (2002), Fajtatarsitas a gyumolcsultetvenyekben. Mezogazda Kiado, Bp. 72–150.

Soltész, M., (2003). A termeskotodes tenyezoi es merteke In: Papp, J. (ed.) (2003): Gyumolcstermesztesi alapismeretek. Mezogazda Kiado, Bp. 306–311.

Southwick, S. M., and Davenport, T. L., (1986). Characterization of water stress and low temperature effects on flower induction in Citrus, Plant Physiol. 81:26-29.

Spann, T. M. and Schumann, A. W., (2013). Mineral Nutrition Contributes to Plant Diseases and Pest Resistance, University of Florida, IFAS extension, https://edis.ifas.ufl.edu/hs1181

Stintzing, F. C. *and* Carle, R., *(*2004*).* Functional properties of anthocyanins and beta-lains in plants, food, and in human nutrition, *Trends Food Sci Technol 15:19–38.*

Stösser, R. (2002). Von der Blute zur Frucht In: Link, H. (ed.) (2002): Lucas' Anleitung zum Obstbau. Eugen Ulmer GmbH Co., Stuttgart. 29–37

Tafinta, I. Y., Shehu, K., Abdulganiyyu, H., Rabe, A. M. and Usman, A., (2013). Isolation and Identification of Fungi Associated with the Spoilage of Sweet Orange (*Citrus Sinensis*) Fruits In Sokoto State, *Nigerian Journal of Basic and Applied Science,* 21(3): 193-196, DOI: http://dx.doi.org/10.4314/njbas.v21i3.4

The Citrus Industry, (2016). http://websites.lib.ucr.edu/agnic/webber/Vol1/Chapter4.html, date retrieved 20/04/16

Tollig, B., Van der Merwe, L. L. and Schutte, G. C., (1996). BAS 490 F: A new fungicidal strobilurin for the control of citrus black spot. *Proceedings of the International Society of Citriculture* 1: 369-372.

Truter M., Kotzé J.M., Janse van R.T.N. and Korsten L., (2004). A sampler to determine available *Guignardia citricarpa* inoculum on citrus leaf litter. *Biosystems Engineering* 89: 515-519.

Uchida, R., (2000). Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms, College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa

UC-IPM (University of California Integrated Pest Management), (2016). http://www.ipm.ucdavis.edu/PMG, date retrieved 20/04/16

Ward, D. L., (2004). Factors Affecting Pre-harvest drop of apple, Ph. D. Dissertation, Virginia Polytechnic Institute and State University

Webb, D., (2014). Anthocyanins, *Today's Dietitian*, Vol. 16 No. 3 P. 20

Webber, J. H., Reuther, W. and Lawton, H. W., (2006). History and Development of citrus industry. In: Reuther, W., Webber, J. H. and Batchelor, L.D. (esd), the citrus industry. University of California Press, Riverside, pp 1-39.

Whiteside, J. O., Garnsey, S. M. and Timmer, L. W., (1988). Compendium of citrus diseases, The American Phytopathological Society Press, St. Paul, MN.

Whiteside, J. O., (1965). Black spot disease in Rhodesia, *Rhodesian Agricultural Journal*, 63, 87-91.

Xu, Q., Chen, L. L., Ruan, X., Chen, D., Zhu, A., Chen, C. and Bertrand, D., (2013). The draft genome of sweet orange (*Citrus sinensis*), *Nature Genetics* vol. 45, No. 1, pp. 59-68

Zekri M. and Obreza, T. A., (2015). Macronutrient Deficiencies in Citrus: Nitrogen, Phosphorus, and Potassium, University of Florida: IFAS extension, retrieved from http://edis.ifas.ufl.edu/ss420

APPENDIX I

Kwame Nkrumah University of Science and Technology

Faculty of Agriculture: Dept. of Horticulture

Questionnaire on Citrus Pre-harvest Fruit Drop in Major Citrus Growing Areas in Ashanti Region

The purpose of the survey is solely to find the status, farmers' perception and their management practices on Citrus Pre -harvest fruit drop. Any information given would be kept confidential and would be used for research purpose only. Please feel free to answer the questions.

Name:……………………………………District…………………Town/Village……

Sex……… GPS………………..

1. Level of education a) No formal education b) Primary c) JHS/middle school d) secondary e) tertiary
2. How many years have you been into citrus farming? A 3-5 years b 6-10 years c. 11- 15 years d. 16 – 20 years e. above 20 years
3. Which variety do you cultivate?

   a. Late Valencia

   b. red blood

   c. ortanique

   d. both late Valencia and red blood

   e. Washington navel

   f. Any other (Specify)…………….
4. What is the planting distance used on your farm? a. 6 m x 6 m. b. 8 m x 8 m

   c. 7 m x 7 m d. 8 m x 7 m e. Any other (specify) …………..
5. How often do you weed the farm? i. Three times a year ii. Twice a year

iii. Not at all

6. What are the problems you encounter in the production of oranges?

…………………..…………………………………………………………………

………………………………………………………………………………………

7. Have you contacted Extension officers on any activity undertaken? Yes/No

8. If you have what kind of concern/problem made you do that?

……………………………………………………………………………………

8. How many acres of citrus farm do you have? ....…………………

9. Do you know of falling of mature fruits (pre-harvest drop) in your citrus farm? Yes/No

10. Which period of the year does it mostly occur? ………………………………….

11. What do you think might be the possible cause(s)? …………………………..

12. Do you suspect insects or fruit flies cause the pre-harvest drop or can play a part? Yes/No

13. What do you think brings about the insect or fruit fly occurrence?

……………………..…………………………………………………………..

14. Around which period of the year do you experience/see the fruit fly or insect?

……………………………………………………………………………………….

15. What do you think are the damage caused on the fruits?

....………………………………………………………

16. What attempt do you make to control the fruit fly or insects?

.....................………………………………………………………………………

17. Have you fertilized your citrus farm since it started bearing fruits? i Yes ii No

18. If yes, how frequent do you fertilize i Once a year ii once every two years

19. Which kind of fertilizer do you use? ……………………………………………….
20. If the answer for question 20 is no, why?……………………………………….

21. Do you suspect a disease plays part in causing the falling of mature citrus fruit?

Yes/No

22. What do you do to control it? …………………………………………………

23. If it is chemical you use, when do you spray? ……………………………………

24. Do you spray or apply the chemical when disease symptoms are visible or can be seen? Yes/No

25. If the answer to question 24 is yes, what was the result? i nothing changed ii there was reduction of the falling of fruits iii Don't know iv. Drop continued

26. Have you pruned your citrus orchard before? Yes/No

27. How often do you pruned? i. Once a year ii. Once every two years iii. Not yet

Any comment(s) ……………………………………………… ……………..

APPENDIX II

Morphological Growth and Spores of *Phyllosticta citricarpa*

The following plates show the morphological growth of the pathogen, *Phyllosticta citricarpa* on the 7th and 14th day of culturing that has been associated with citrus black spot. a.

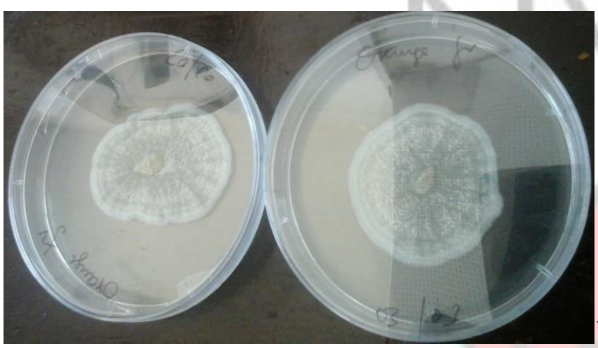

b.


Figure 5.2: a). Front view and b). Back view of morphological growth of *Phyllosticta citricarpa* on the 7th day

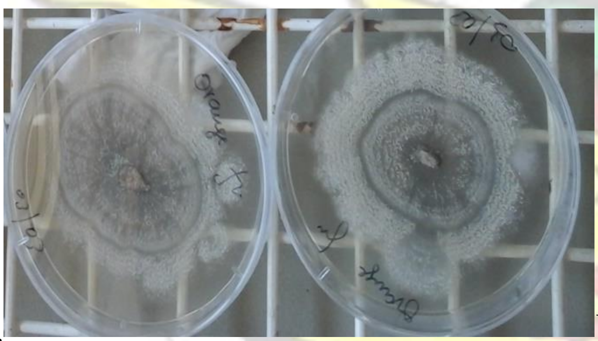
a.

b.


Figure 5.3: a). Front view and b). Back view of morphological growth of *Phyllosticta citricarpa* on the 14th day

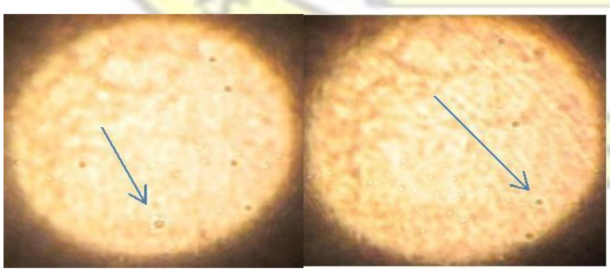


Figure 5.4: Spores of *Phyllosticta citricarpa*