RESPONSE OF AVOCADO (*Persea americana* Mill.*)* ROOTSTOCKS TO SOIL FLOODING AND INOCULATION WITH *Phytophthora cinnamomi*

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BOTANY (MICROBIOLOGY)

DEPARTMENT OF BOTANY

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DECLARATION

I certify that this thesis has not been previously presented for a degree in Maseno University or in any other University. The work reported herein is my original work and all sources of information have been supported by relevant references.

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ACKNOWLEDGEMENT

This work has been made possible by various individuals, whom without their assistance and guidance I would have not reached this far.

I deeply appreciate the work of my supervisors, Dr. David M. Musyimi and Dr. George Opande who have guided me all through from the beginning of my proposal writing, data collection and analysis up to thesis writing. I also thank the fruitful help provided by Mr. Mmayi Millar Patrick during data analysis and interpretation, for he spared all his work and offered me assistance and knowledge on the same.

My gratitude and sincere thanks also goes to the National Council of Science and Technology (NCST) which funded this project. Thanks to the teaching and non-teaching staff of the department of Botany, Maseno University who have made my work easier and assisted during course work stage.

My special thanks are also extended to my family members, especially my father Emmanuel Nikasio Shiranda and mother Philomen Nafuna Shiranda for their constant financial and moral support throughout my studies. I will not forget my sister Bridgit Shiranda in a special way for her support and encouragement.

I would like to extend my gratitude to my husband Antony Wabuti who has been there at point of support and gave enough time when need arose to be in Maseno University for my work.

DEDICATION

This work is dedicated to my family. Through the years I have learned how love, friendship, sacrifice and unity in a family can be a strong base of commitment, inspiration and accomplishment. Highly dedicated to my very own daughter Anneshirleen Wabuti and son Deogratias Shiranda Wabuti, whom I really pray, guide as they grow and hope that they learn to fulfill dreams that are almost equal to mine.

ABSTRACT

Avocado plants (*Persea americana* Mill.) belongs to the family lauraceae. Avocado fruit has high amounts of fats and proteins, high dietary fibre, vitamins and potassium. It is known to be the most nutritious of all the fruits. The most devastating pathogen of avocado plants is a fungal species, *Phytophthora cinnamomi* which cause avocado root rot. The fungus is the most widely distributed of *Phytophthora* species. It affects growth and physiology of plants and even leading to death of plants. When this pathogen is combined with flooding, the potential for asphyxiation leading to root decay and death is increased. Even though several studies have evaluated the tolerance of different avocado rootstocks for tolerance to *Phytophthora cinnamomi* there are few such studies in Kenya which have characterized the pathogen and evaluated the effect of the pathogen on the physiology of avocado rootstocks under flooding conditions. The objectives of this study therefore were to isolate and morphologically characterize *P. cinnamomi*, and investigate growth and physiological responses of avocado rootstocks to *P. cinnamomi* under water logging conditions. Four avocado rootstocks (Puebla, Fuerte, Booth7 and Pinkerton) were obtained from orchards in Maseno, Nyando, Kisumu East and Busia-Budalangi of Western Kenya. The pathogen was isolated from the soil through plating on selective medium and by baiting methods. Ripe avocado fruits were used as baits. Morphological characterization of the pathogen was carried out. Avocado rootstocks were planted in 10 litre plastic pots containing sand - soil mixture of ratio 1:2 for 3 months and then inoculated with *P. cinnamomi* under flooded and non-flooded conditions. The plastic pots grown with plants were laid out in a greenhouse in a completely randomized design. Growth was assessed by determining the plant heights, stem diameter, plant fresh weights, plant dry weights, and leaf area. The seedlings were uprooted at the end of the study for root necrosis examination. Data on chlorophyll concentration and chlorophyll fluorescence parameters were collected using a spectrophotometer and portable fluorescence monitoring system respectively. The data collected was subjected to analysis of variance. Treatment means were separated and compared using least significant difference at 0.05. Flooding and inoculation with *P. cinnamomi* significantly ($p \leq 0.05$) reduced the growth of the avocado rootstocks. This was reflected in the reduction of plant height, stem diameter, leaf area and dry weights. Disease incidence led to reduced plant growth and massive death of avocado rootstocks such as in Fuerte and Pinkerton under flooded and inoculated conditions. Significant reductions chlorophyll *a, b* and total chlorophyll concentration were also evidenced from this study. Generally Fv/Fm, ΦPSII and ETR of the four avocado rootstocks decreased under flooding and *P. cinnamomi* inoculation treatments, an indication that photosynthetic apparatus were affected contributing to the reduction in avocado growth. A significant interaction was found between the various treatments and avocado rootstocks as far as plant height, plant stem diameter, plant fresh and dry weights, disease incidence, chlorophyll *a, b,* total chlorophyll concentration, and chlorophyll fluorescence parameters. Out of the four selected rootstocks studied, Puebla rootstocks responded better to flooding and *P. cinnamomi* inoculation, and therefore may be recommended for growing in flood affected regions of Kenya where *P. cinnamomi* infestation is also a common problem.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA: - Analysis of Variance

Chl a: - Chlorophyll *a*

chl a+b: - Total chlorophyll

chl b: - Chlorophyll b

DAI: - Days after inoculation

DNA: - Deoxyribonucleic Acid

DSP: - Double Super Phosphate

ETR: - Electron transport rate

FV/FM: - Maximum quantum yield

ICRAF: - International Centre for Research and Agroforestry.

MOA: - Ministry of Agriculture

NPQ: - Non potential quenching

PAR: - Photosynthetic Active Radiation

ϕPSII - Effective quantum yield

PRR: - Phytophthora Rands

trt: - Treatments

Var: - Variety

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Avocado (*Persea americana*) industry is based on seedling rootstocks. Seedling rootstocks are chosen according to their ease of propagation but the horticultural value of rootstock is not studied (Coffey, 1992). In many countries seed propagated rootstock are still chosen according to availability and nursery performance rather than as to orchard performance (Allen *et al*., 1980). After a new rootstock clonal propagation method was developed in California it was spread to Israel where green ovacado cutting mist spray was developed. Unfortunately the methods never became commercial (Baum and Pinkas, 1988).Avocado is an evergreen tree, large size of the trees of most commercial cultivars cause excessive expenses for management and overcrowding in orchards. Growing dwarf cultivars means easy management, but not of commercial success (Reeksting *et. al*., 2014). Therefore, it should be understood that rootstock effects on tree size and vigor is strongly related to tree productivity. Frequently, soil stress factors act together, sometimes synergistically, such as rot rot and poor aeration or sometimes antagonistically, such as root rot and lime (Ben-Ya`acov and Esther, 1995). The breeders should therefore, take into account actual combinations of stress factors and select rootstocks for them and not for an individual factor. Avocado is highly susceptible to *P. cinnamomi* and is a commercially valuable fruit tree cultivated in tropical climates throughout the world, producing a green-skinned, pear-shaped fruit that ripens after harvesting (Menge *et al.*, 2012). They occupy small areas, but give good yields which fetch high prices when compared to other field crops. This combination of high yield and high prices in heavily populated areas like in the Lake Victoria Basin can increase food security of households. Avocado became a relatively new Kenya`s commercial fruit crop in 1980`s as reported by Njuguna (2005). Earlier before it became commercial, it was planted in home gardens in its

countries of origin that include Mexico, Colombia and Ecuador (Pandey *et al.*, 2010). According to Menge *et al.* (2012) among other factors of avocado industry, rootstocks are more important. More so, avocado tree development, health and productivity in fruits are dependent on rootstock type (Christie, 2012). Avocado rootstock research is known to have developed very slowly and became a major subject of research in California when root rot disease caused by *Phytophthora* became important (Dinis *et al.*, 2011). In Kenya, there is slow rate of increase on avocado rootstock research and that a lot of damages are being caused by the pathogen *Phytophthora cinnamomi* (Gimeno *et al*., 2012). Also, no clear method of elimination has been discovered to be functional in Kenya (Colmer and Voesenek, 2009).Much work has been done on the tolerance of avocado rootstocks to environmental stresses, but little has been done combining two or more stresses together such as flooding and *Phytophthora cinnamomi* inoculation.

The production of avocado in many cases is negatively affected by factors such as reduction in soil fertility, poor agronomic practices and lack of healthy seeds (Arpaia *et al*., 1993). Root rot disease of avocado is ranked the first devastating disease of avocado that is caused by *Phytophthora cinnamomi* (Reeksting *et al*., 2014). This disease has led to reduction in avocado production in most avocado producing countries (Randy *et al.*, 2001). In Kenya the current production of the fruit cannot cope with the ever-increasing human demand and this is partly due to the infection of the tree by *Phytophthora cinnamomi*. Although Kenya has 7,500 ha under avocado cultivation that yields of about 81,000 tons, about 30,000 to 40,000 tons is lost due to poor pre and postharvest handling practices. These poor handling will initiate *Phytophthora cinnamomi* infection to plants. Limited root rot resistance varieties, poor avocado tree crop management practices, poor market information, pests (thrips, scales, fruit fly and systates weevil) and diseases (root rot, anthracnose and *Cercospora* leaf spot), and limited utilisation of avocado (MoA, 2005) also attribute to these low yields.

In regions of Lake Victoria Basin of Kenya, avocado production has expanded to swampy areas that have soils that drain slowly (MoA, 2005). This combined with poor irrigation design and management has increased the potential for flood-induced root asphyxiation leading to root decay due to *P. cinnamomi* (EPPO, 2004). The root rot caused by *P. cinnamomi* Rands, a soil borne organism that is initiated to spread by flooding as water spreads motile zoospores that are chemotactically attracted to, infect, and kill root tips of avocado (Randy *et al*., 2001). This makes it most severe in poorly drained and flooded soils with asphyxia conditions. In avocado trees, root asphyxiation has resulted in a delay of vegetative spring shoot growth, a reduction in fruit yield (40%), reduced biomass accumulation, loss of foliage and tree death that is associated with reduction in plant chlorophyll, chlorosis and reduction in photosynthetic apparatus performance (Gil *et al*., 2007). It is not known the extent of avocado losses due to *P. cinnamomi* in Kenya, but, annual losses in USA have been estimated at 30million. There are limited studies that have determined the effect of flooding and *Phytophthora cinnamomi* inoculation on chlorophyll concentration and chlorophyll fluorescence.

Phytophthora cinnamomi is a soil-borne microbial pathogen that causes root rot and crown rot of many horticultural roots of ornamental and forestry crops (FAOSTAT, 2004) It preferentially attacks "feeder roots". It is the most widely distributed *Phytophthora* species with over 1000 species (Whiley *et. al*., 2002) that is mostly found causing significant root rot avocado loses. Propagules of the pathogen spread by soil movement, including wind-blows or debris, or by water flow and runoff in drainage/irrigation ditches. There is therefore need to determine *Phytophythora* (PRR) resistant avocado rootstocks. For instance, Menge *et al*. (2012) worked with three tolerant varieties `Steddom` (PP24), Zentmyer` (PP4) and Uzi(PP14) and found Steddom and Zentmyer to be resistant to *P. cinnamomi* in America .However, there is need to check the responses of varieties commonly grown in Kenya.

Approximately a third of farmers in Kenya grow avocado (FAOSTAT, 2004) and 40 - 50% of the population consumes avocados regularly. Avocado is mainly grown in Western, Nyanza and Rift valley regions, but unfortunately some areas of Nyanza e.g. Nyando (Okeyo *et al*., 2008) where avocado is grown are water logged and infected with *Phytophthora cinnamomi*. Much more badly, the cure of avocado root rot has not yet been identified in most farming regions in Kenya and even other countries (FAOSTAT, 2004). Based on this reason, there is need to advise farmers to use several integrated measures to reduce the spread of disease to the unaffected regions and use varieties that are tolerant to the pathogen. The disease causes great losses because after infection, the plant withers and dies. In addition, the fruits drop from the tree before maturity (MoA, 2005). The control measures that are currently in practice include; use of clean nursery practices, use of fungicides, cultural practices and biological methods (Griesbach, 2005). However, these measures have not completely solved the problem of occurrence of root rot of avocado. They are expensive for most farmers and some are harmful to the environment e.g. use of fungicide. It is therefore necessary to embark on research, which would bring solution to this devastating disease of avocado. This includes introducing a tolerant variety that would be able to resist the pathogen from thriving within the plant to cause root rot.

According to Reeksting *et al*. (2014), control strategies for avocado root rot include phosphonate trunk injections, development and use of tolerant rootstocks, and proper orchard management practices including use of pathogen-free material and prudent irrigation scheduling. Irrigation and soil water content are particularly important factors to consider when avocados are grown under flooding and P. *cinnamomi* (Reeksting *et al*., 2014).There are limited studies in Kenya that have focused on physiological responses of avocado rootstocks to flooding and *Phytophthora cinnamomi* stress.

Waterlogged or flooded soils in lake Victoria regions of Kenya may result from high rainfall, river overflow, elevated water tables, inadequate drainage and improper irrigation management as it occurs in other areas (Colmer and Voesenek, 2009; Pandey *et al*., 2010). Avocado growth is sensitive to flooding and *Phytophthora* root rot (PRR) and therefore tolerant rootstocks need to be investigated and identified for recommendation for growth in these regions of Kenya.

The world's production of avocado in 2004 was 3.2 million tonnes (ICRAF, 2007) that included a major contribution from North and Central America. Kenya was ranked 6th in export value of the avocado in the world (FAOSTAT, 2004). About 85% avocado produce in Kenya was by small holder-farmers. Avocado crop is therefore an important crop to rural communities and economies (Cooper *et al*., 2003). Despite the favourable climate in the Lake Victoria Basin, production of avocado in this region is still low and this is due to the constraints such as diseases, salinity and water logging (Schaffer *et al*., 1992).

In Kenya, avocado cultivation is concentrated on the highlands between 1,200 and 1,800 m (ICRAF, 2007). In these areas avocado is grown in several agro-ecological zones mainly by small-scale growers (85%) who grow it for subsistence, local markets and export (Cooper *et al*, 2003). About 70% of avocado is grown in Central and Eastern parts of Kenya. Central regions produces 40%, Eastern 28%, Western 13%, Rift Valley 10%, Nyanza 6% and Coast 2% and Nairobi county 1% (MoA, 2005).

Flooding inhibits root growth, shoots and knew leave development, reduce net photosynthetic rate, photosynthetic electron transport rate, photosystem II photochemical efficiency and cause reactive oxygen species metabolism disorder (Reeksting *et al*., 2014). Some plants sense oxygen levels and bring morphological, physiological and biochemical changes that improve flood tolerance and also reduce in carbohydrate consumption. Zebin *et al*., 2014 while

researching on restoration of *Distylium chinense*, ashrub after a damconstruction, found it to maintain stable P_n. A decrease in Fv/Fm, qP and ETR accompanied by an increase in qN have found to reflect increased photoprotection through the xanthophyll cycle rather than photo-damage (Zebin *et al*., 2014). It is therefore necessary to determine chlorophyll fluorescence parameters, in order to determine if avocado rootstocks have a stronger adaptability to soil flooding, which may be a factor to enable them survive in flooded areas. Much more interesting, other regions with flooding have been found to be habitats for *P. cinnamomi.* Morphological characterization and identification has not been carried out on this pathogen, therefore there is need to carry out morphological characterization and identification of the *P. cinnamomi* strains attacking avocado rootstocks locally.

1.2 Problem statement

Root rot caused by *Phytophthora cinnamomi* has continued to be a menace in most avocado producing regions in Kenya. This has led to poor yields in Lake Basin regions that are often under flooded-asphyxia conditions (Okeyo *et al*., 2008). Tedious efforts have been made to increase avocado fruit production by extirpating *P. cinnamomi* infected avocado rootstocks or treating with fungicides but still no remarkable success (Griesbach, 2005).Morphological characteristics of avocado plants under flooding and *P. cinnamomi* inoculation in lake region of Kenya have not been studied, and little is known on the effect of *Phytopthora cinnamomi* inoculation and flooding on the growth and disease incidences of avocado rootstocks commonly grown in Kenya. Infection of avocado by *P. cinnamomi* poses a big problem as it has caused a lot of losses in avocado producing regions in Kenya. Knowledge pertaining to the physiological and growth tolerance of avocado rootstocks such as Puebla, Fuerte, Pinkerton and Booth7 to *P. cinnamomi* and flooding is limited. The effect of the fungus on the general growth of the avocado root stocks have not been conclusive and little is understood on the effect of *Phytopthora cinnamomi* inoculation on chlorophyll content and chlorophyll fluorescence of

avocado rootstocks under flooding conditions in Kenya. It is therefore prudent to further investigate growth parameters of common avocado rootstock in Kenya, with a view to identify the tolerant ones to disease incidences. There is also limited scientific initiative that has documented on the morphology and physiology of avocado rootstocks in Kenya under flooding and *P. cinnamomi* inoculation. Avocado plant is a multipurpose tree which has sustained human nutrition, and has received scanty attention through research despite its potential to alleviate poverty, malnutrition and contribute to food security in Kenya. As Kenya's population continue to rise, there is pressure on land for diversified food production and increased yield, hence the need to evaluate the response of avocado rootstocks to soil flooding and *P. cinnamomi* inoculation.

1.3 Justification of the research problem

Avocado fruits are importantly as they contain high level of proteins, therefore very nutritious with 20 different vitamins and minerals. They do not contain any cholesterol and have very low saturated fat. Owing to the importance of avocado in the local market, there is urgent need to expand avocado production in Lake Basin waterlogged regions. Most parts of Kenya are arid and semi-arid thus, avocado growth becomes difficult due to need for irrigation. To increase food security and raw avocado to industries, further expansion of avocado production in Kenya that may call for growing it in perennially flooded areas and wetlands that include lake basin water logged areas of Nyanza that are infested with *P. cinnamomi*. These efforts to expansion cannot succeed because of water logging and *Phytophthora* infestation in these areas, and therefore need to have rootstocks that are resistant to these abiotic and biotic stresses (Pegg *et al*., 2002). With the current shrinking of arable land, avocado plants are being grown in potentially flooded areas such as the Lake Victoria region. There is need for research work to select rootstock varieties that can survive well in waterlogged areas and those that are prone to diseases caused by pathogens such as *P. cinnamomi*.

There are also many species of *Phytophthora* that causes root rot in avocado trees and therefore morphological identification will confirm of *P. cinnamomi*. This study is very important because it intends to contribute suitable ways in which lose of avocado due to root rot disease can be controlled by planting resistant rootstock varieties. This is essential because the most common ways that have been recommended by scientists such as, use of clean nursery practices, disinfecting of soils, use of fungicides, cultural practices and biological methods have not been able to fully eliminate effects of the disease (Fleischmann *et. al*., 2002).

1.4 Objectives of the study

1.4.1 General objective

To evaluate selected avocado rootstocks commonly found growing in the Lake Victoria Basin of Kenya for response to water logging and *Phytophthora cinnamomi* inoculation under green house conditions.

1.4.2 Specific objectives

i) To morphologically identify *Phytophthora cinnamomi* of avocado rootstocks commonly found growing in Lake Victoria basin of Kenya.

ii) To determine the effect of *Phytophthora cinnamomi* inoculation on growth of avocado rootstocks seedlings growing under water logging conditions.

iii) To determine the effect of *Phytophthora cinnamomi* inoculation on disease incidence of avocado seedlings grown under water logging conditions.

iv) To determine the effect of *Phytophthora cinnamomi* inoculation on chlorophyll concentration and chlorophyll fluorescence parameters of avocado rootstocks seedlings grown under water logging conditions.

1.5 Hypotheses

i) There are no differences in morphological characteristics of *Phytophthora cinnamomi* of avocado plants grown in the Lake Victoria basin of Kenya.

ii) *Phytophthora cinnamomi* does not reduce growth of avocado seedlings in waterlogged conditions.

iii) *Phytophthora cinnamomi* does not increase disease incidence of avocado seedlings in waterlogged conditions

iv) *Phytophthora cinnamomi* does not reduce leaf chlorophyll concentration and chlorophyll fluorescence parameters of avocado rootstock seedlings in waterlogged conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 The avocado species identity and distribution

Persea americana (Miller) is the current scientific name under the family *Lauraceat*.Other synonyms include: - *Haurus persea* L. *Persea drymifolia* (Schlecht & Chm) (ICRAF, 2007). Some common names include: - alligator pear and avocado in English, avocat in French, apukado in Malay, aguacate in Spanish, Alligatorbirne in German and Mparachichi, mpea or mwembe mafuta in Swahili. Medang is the trade name (ICRAF, 2007).

Avocado is a tree with 9 - 20m in height classified generally as evergreen with leaves that vary in shape at length of 7 – 41cm. Inflorescence is borne on terminal position. The fruit is a berry, consisting of a single, large dicotyledonous seed, surrounded by a buttery pulp. The fruit contains 3 – 30% oil with skin`s thickness and texture that vary. Fruit colour at maturity is green, black or reddish depending on variety. Fruit shape ranges from spherical to pyriform and weighs up to 2.3kg depending on the variety (ICRAF, 2007).

The probable area of origin is the Chiapas, Guatemala, and Honduras center and found its way to the rest of Southern and Central America (Njuguna, 2005). It is exotic to all African countries with suitable growing conditions like Kenya. It was introduced to Kenya in 1930`s (ICRAF, 2007). It is also now grown in India, Indonesia, Thailand and Vietnam among others. In Kenya it is grown in Murang'a, Thika, Kakamega, Trans Nzoia, Meru, Machakos, Embu, Nyeri, Taita Taveta, Nyamira, Kisii and Vihiga (Njuguna, 2005).

2.2 Avocado varieties and growth conditions

There are several varieties of avocado. These include; Bacon, Duke, Anaheim, Ganter, Jim, Lula, Lyon, and nabal, Puebla, Pinkerton, Gueen, Reed, Sama, Tambarina and Winter Mexican (Menge *et al.,* 2012). There are two major varieties grown in Kenya, namely Fuerte and Puebla (Njuguna, 2005). Fuerte's is green even when ripe and mostly oval shaped. Its inside is creamy to pale green and is sweet to taste. It grows to a large or medium size. (Njuguna, 2005). Puebla is round in shape and turns to purple when ripe. It`s flesh is creamy in color and the variety is available in Kenya throughout the year (ICRAF, 2007).

Avocado is grown in altitudes from 1300 to 2100m above sea level. They require well-distributed rainfall of 1200mm for optimal production with a minimum 700mm. Avocados thrive in temperatures between 14^{o}C and 24^{o}C and high temperatures above 30^{o}C destroy the fruit (Menge *et al.,* 2012). *Persea 11ertilize* requires well aerated soils preferably loam soils. Water logging affects the crops, as roots are intolerant of anaerobic conditions. The optimal soil pH for avocado ranges 6.0 – 8.0 (ICRAF, 2007).

2.3 Avocado plant propagation, management and yield

Germination is hypogeal although most varieties can be natured through vegetative propagation. However, they are first planted in nurseries as seedlings before being transported to the field (Njuguna, 2005). Grafting of terminal leafy shoots or of buds as scion material onto young vigorously growing seedling roots 4 months old is common (Pandey *et al.,* 2010). Planting fields are well prepared before rainfall. Disease and pest free plants are planted into 60cm by 60cm by 60cm planting holes at 9m by 9m by 9m spacing in soil mixed with manure and 120gm of Double Super Phosphate (DSP) or Triple Super Phosphate fertilizer (Reeksting *et al*., 2014). Regular weeding, mulching and pruning are carried out. Intercropping may be done with other crops such as peas, cabbages in the first three years when the trees are still small but

discontinued when trees grow big. Commercially after several years of production, it is desirable to occasionally reduce canopy width of trees to 5 – 6m to reduce spraying and harvesting costs and reduce storm damage (Randy *et al*., 2001).

Fruit production from budded or grafted trees is within 2 or 3 years as compared to the 8-10 or more years required of seedling avocado depending on variety (Reeksting *et al*., 2014). Some varieties do not change colour on maturity. The yield of avocado tree ranges from 230 to 320kg of fruits per tree per year. Trees of age 3 – 5 years will yield 300 – 400 fruits per tree while those above 5 years will yield 800 – 1000 fruits per tree. The main avocado harvest season in Kenya is from March to September (Njuguna, 2005). The fruit does not generally ripen until it falls or is picked from the tree. Fruits are cut with about 3cm stalk on them using a ladder soon after which the fruits are cooled to optimum storage temperature of 5 ^{o}C for the major cultivars (ICRAF, 2007).

2.4 Functional uses of Avocado

Avocado has an edible flesh representing 60 – 75% of the total fruit weight. It provides a nutritious tasty solid food even for infants (Pegg *et al*.**,** 2002). It is consumed uncooked as cooking impairs flavour and appearance. Each 100g edible portion contains about 65 – 86g water, 1- 4g proteins, 5.8 – 23g fat (Mono-saturated and anti-cholesterol agent), 3.4 – 5.7g carbohydrates 0.8 – 1g iron, 1.5 – 3.2mg of vitamins A and B also rich in vitamin E. The energy value per 100g is 600 – 800KJ (ICRAF, 2007). Avocado fruit have been used as surplus fodder for farm animals such as pigs (MoA, 2005). Avocado depends on large insects such as bees for pollination. Many avocado farmers are advised to keep hives to serve this purpose, which also promotes honey production (Cooper *et al*., 2003).

Avocado wood is still useful in house building, furniture making, carving, and small articles such as pen and brush holders; although timber from avocado is brittle and susceptible to

termite attack and that avocado are more seldom produced for their fruit rather than their wood (FAOSTAT, 2004). The pulp and seed contain fatty acids such as olive, lanolin, palmitic, stearic and others that constitute 80% of the fruits fatty content. The oil is used by the cosmetic industry in soaps and moisturizer products (ICRAF, 2007).

Recent researches show that extracts of leaves and fresh shoots of avocado have anti-cancerous activity (Whiley *et al*., 2002). Oil extracts of seeds have astringent properties and oral infusions of the leaves have been used to treat dysentry (ICRAF, 2007). Furthermore, avocado fruit epicarp has anti-helmintic properties (Griesbach, 2005). Ground seed is made into an ointment used to treat various skin afflictions such as scabies, puruheat, wounds and scalp lesions and dandruff (ICRAF, 2007). The unripe fruits are poisonous and the ground up seed mixed with cheese has been used as a rat and mouse poison (ICRAF, 2007). In Kenya avocado is the leading exported fruit that is mainly exported to Europe (Reeksting *et al*., 2014). On the European Market, Kenya competes with Israel, Spain and Mexico (Stewart *et al*., 2012). Although on the local scenes in Kenya, there is no organized structure as farmers, buyers and consumers operate independently (Mwai, 2001).

2.5 The growth and reproduction of *Phytophthora cinnamomi*

Phytophthora cinnamomi is a water mould, microscopic organism previously classified as a fungus. The scientific name *Phytophthora* (pronounced fy-TOFF-thor-ah) is derived from the Greek words meaning 'plant destroyer' **(**Jeffers and Sisco, 2009). The pathogen was identified as the cause of death of cinnamon trees in Sumatra in 1921; hence its former common name 'Cinnamon fungus (Randy, 2001). The genus *Phytophthora* includes a number of serious pathogens including *Phytophthora infestans*, the cause of the potato famine in Ireland in the1840s (FAOSTAT, 2004). Based on historical evidence of agricultural impacts in the 1800s, the pathogen is believed to have been brought into Australia by early European settlers, presumably within infected plants. First detected in Australia in 1935, *Phytophthora cinnamomi* has since been introduced to many locations across the Australiancontinent. It has contaminated thousands of hectares in Western Australia, Victoria, Tasmania and South Australia, as well as wet coastal forests in Queensland. Rainfall, temperature and soil characteristics (type and structure) have influenced the geographic distribution of *Phytophthora cinnamomi* (Reeksting *et al*., 2014).

Infection of plants by *Phytophthora cinnamomi* is favoured by free water in the soil or ponding on the soil surface. Warm wet soils, especially those with impeded drainage, favours the germination of *P. cinnamomi* chlamydospores. These spores enable the pathogen to survive in an area during harsh environmental conditions. This because they provide long distance spread just in case contaminated soil or dead plant materials are moved. As the roots of the infected plant die, long-lived resistant chlamydospores are produced again. This provides a source for future re-infection of seedlings in the area (Gil *et al*., 2007).

2.6 Mode of infection of *Phytophthora cinnamomi*

Local and seasonal variations in environmental conditions influence the virulence of *P. cinnamomi*. For instance, if environmental conditions for *P. cinnamomi* are not optimal, despite its presence, it may not be active (Van Rooyen, 2011). Soil moisture levels and temperatures significantly influence the activity of *P. cinnamomi*. Temperatures between 15 °C and 36 °C accompanied by high soil moisture levels due to rains favours the production of free swimming spores. This increases their potential for infection due to the spread of spores downhill (Colmer and Voesenek, 2009). Plants with damaged roots will naturally succumb more rapidly, especially with greater sun exposure. Mountain Ash (*Eucalyptus regnans*) is one of important timber trees, that is susceptible to *P. cinnamomi*. However, the combinationof cold winters, dry summers and high organic matter in the soil act to safeguard plants against *P. cinnamomi* (Dinis *et al.*, 2011)

Chlamydospores germinate to produce sporangia that release motile spores (zoospores). Zoospores are able to locate and attach to root tips after which they give rise to fine filamentous threads (hyphae) that may invade the roots of the host (Christie, 2012). However, there is variation in the response of hosts to the pathogen. Some hosts may show no obvious symptoms due to the ability to curtail spread of the pathogen within their tissue (Corcobado *et al*., 2013). Such plants are said to have low susceptibility. In highly susceptible host species, P. *cinnamomi* hyphae spread throughout the root system until they girdle the major roots and stems. It impedes the ability of plant's vascular tissues to absorb nutrients and water. This leads to symptoms in some larger plants that are alike to those of drought stress (Gil *et al*., 2007**).**This includes outermost parts of vascular tissue becoming yellow and dying first ('dieback'). The expression of dieback has been mostly limited to Ash species [e.g. Silvertop Ash (*Eucalyptus sieberi*)] and stringy bark species of low timber productivity coastal forest in east and south Gippsland (Pegg *et al.*, 2002**).** Warm wet summers followed by warm dry autumns that favour *P. cinnamomi*

development. Water-logged conditions in plants, has led to epidemic outbreaks of *P. cinnamomi* in this forests, as well it becomes worse as these situations tends to be cyclic (Kong *et.al,* 2003).

2.7 Biology and Geographic distribution of *P. cinnamomi*

Sporangia is released into soil water and it swims to small roots due to chemotactic response to root exudates, encysts and germinate on the root surface. Penetration occurs within 24 hrs of germination (Christie, 2012**)**. The fungus then spread in the young feeder roots causing a rot which may extend into the base of the stem, propagules may also be flashed onto and infect aerial parts of the plants (Van Rooyen, 2011). *Phytophthora cinnamomi* survives in dead plant material and can survive for long period in this substrate. This is a saprophytic phase that can allow an increase in population of the pathogen **(**Colmer and Voesenek, 2009). *Phytophthora cinnamomi* may also survive in the soil as mycelium, sporangia, zoospore cysts, chlamydospores and oospores and survival can be extended in the presence of an organic substrate (Else *et al.,* 2009). Mycelium of *P. cinnamomi* can survive for at least 6 years in moist soil and zoospore cysts can survive for at least 6 weeks at between -5 and -15 MPas soil matric potential. *Phytophthora cinnamomi* is heterothallic; as oospores are very rare, and are slow to germinate. Varying germination periods may help to maintain a low but continuing population of chlamydospores **(**da Silva *et al.,* 2011). Chlamydospores form in soil, gravel or plant tissue during dry periods, germinate under moist conditions and grow to form mycelia and sporangia or more chlamydospores (Coffey, 1987). The latter may, in turn, remain dormant until conditions become suitable then germinate to produce infective mycelia, sporangia and zoospores or more chlamydospores. This cycle may take at least 5 years provided there is a nutrient source and a non-competitive soil micro flora (Pandey *et al.,* 2010).

The geographical origin of *P. cinnamomi* is not clearly established but it was first described in Indonesia and Sumatra suggests (Mittler, 2006). The species is indigenous in South

East Asia, and in South Africa, and spread across pacific to Latin America in the 18thcentury (Reeksting *et al*., 2014). The appearance of the fungus in the European employment policy observatory (EEPO) region is much more recent (Van Rooyen, 2011).

2.8 Symptoms of root rot caused by *Phytophthora cinnamomi* in Host plants

Early symptoms of infection include wilting, yellowing and retention of dried foliage and darkening of root colour. Infection often leads to death of the plant, especially in dry summer conditions when plants may be water stressed (Whiley *et al.*, 2002). Root infectedrhododendrons and azaleas and tree saplings develop above ground leaf chlorosis, necrosis, wilt, leaf curl, and death. Stem necrosis may not occur for many weeks after the development of wilting symptoms. Below-ground symptoms are most severe in poorly drained soils and include necrosis of young feeder roots and the lower vascular tissues around the crown and just below the soil line (Gimeno *et al.,* 2012). Cankers may become visible at the base of 1-2 year old plants. The roots of older plants may recover from disease and may not develop a canker of the base of the stem. Older plants may remain symptomless, or display only mild `dieback` despite severe root rot (Christie, 2012).

The host range is very wide; *P. cinnamomi* is the most widely distributed *Phytophthora* species, with nearly 1000 host species (Fleischmann *et al.*, 2002). The principal food crop hosts of *P. cinnamomi* are avocados (*Persea americana*), with which the European Union is exclusively concerned (Bergh and Ellstrand, 1986) and pineapples (*Ananas comosus* on which it causes root and heart rot. *P. cinnamomi* also attacks *Castanea*, *Cinnamomum*, *Coniferales*, *Ericaceae* that include *Rhododendron* spp., *Eucalyptus*, *Fagus*, *Juglans*, *Quercus* and many ornamental trees and shrubs(Colmer and Voesenek, 2009). Temperate fruit trees are not important hosts in practice, but in the EPPO region, avocados are a significant host, in the limited areas where they are grown (Dias and Marenco, 2006).

2.9 Disease cycle in avocado

Phytophthora *cinnamomi* lives in the soil and in plant tissues, can take different shapes and can move in water (Steward *et al*., 2012). High water tables and excess irrigation provide suitable conditions for increased zoospore inoculums levels and subsequent root infections (Coyier and Roane, 1986). Thus, excessive soil water increases the incidence and severity of disease (Erwin and Ribiero, 1996). Zoospores are most readily released in soil water matric potentials higher than -5 mbar, or free-standing water (Else *et al.,* 2009**;** Erwinand Ribiero, 1996). Hence, disease is not as common in sandy well-drained soil **(**Else *et al.,* 2009**)**. Once a host is infected, the water flow through the xylem is reduced via wilt-inducing toxins such as ß-glucans and ß-glucan hydrolases. Unlike healthy plants, those infected with *P. cinnamomi* do not recover from the stresses of low soil moisture (Dias and Marenco, 2006). Excessive use of nitrogen-based fertilizers further increases susceptibility to disease due to the increased uptake of water from the soil matrix that favours it`s spread. Dispersal occurs via multiple avenues: ground water, streams, and irrigation, as well as infested potting soil, splash from pot-to-pot, infested pot bases on polyethylene, and diseased nursery stock Dinis *et al*. (2011).

2.10 Isolation and identification of *Phytophthora cinnamomi*

The fungus can be isolated from the soil and plant material either by plating on a selective medium or baiting. Direct soil plating involves suspending 1 g of infested soil in approximately 25 ml of distilled water. The soil to water ratio may be adjusted depending on the pathogen population levels. The soil-water suspension is transferred onto the surface of PARPH-V8 selective agar plates at a rate of approximately 5 ml of suspension per plate, then allowed to incubate for three days. PARPH-V8 contains 20 g agar, 200 ml filtered V8 broth, 800 ml deionized water, 50 g hymexazol, 5 mg pimiricin, 10 mg rifampicin, 250 mg ampicillin, and 125 mg a.i. pentachloronitrobenzene. After a three day incubation period at room temperature, the

residue on the plates is washed off under running tap water with a spatula or finger pads so that the plate can be examined for colonies (Erwin and Ribiero, 1996).

Alternative to direct soil plating, various methods have been developed to bait *Phytophthora* species from the soil, including the use of susceptible plants, leaf pieces, apples, pears, lupine radicals, and pine needles. *Phytophthora cinnamomi* is most reliably detected from fresh soil samples using camellia leaf pieces as bait (Ferguson and Jeffers, 1999). A subsample of the fresh soil is flooded in a deep Petri dish and flooded with 50 ml of distilled water. Five surface disinfested camellia leaf disks are floated in the plate and incubated in the dark for 72 hours. The baits are then removed, blotted on a paper towel, and plated on PARPH-V8 (Ferguson and Jeffers, 1999).

Erwin and Ribiero (1996) showed that sporangia are ovoid, obpyriform with an apical thickening, tapered or rounded at the base, and terminally borne. Sporangia, which release motile zoospores, are not readily produced in axenic culture. Chlamydospores are produced abundantly axenically and from infected tissue. They are borne from hyphae, and globose with thinner walls. Sizes range from 31 to 50 µm in diameter and are either terminal to intercalary in the mycelium (Reeksting *et al*., 2014). The fungus is heterothallic, requiring compatible types to sporulate sexually. Antheridia are amphigynous, averaging 19 x 17 µm. Oogonia are round with a tapered base, smooth, hyaline to yellow, with size ranging from 21 to 58 µm. Oospores are hyaline to yellow, and plerotic and Sizes range from 19 to 54 µm (Steward *et al*., 2012).

2.11 Effects of water logging stress on *P. cinnamomi*

Conditions of high soil moisture are known to favor *Phytophthora* root rot (Corcobado *et al.,* 2013**)**. Earlier studies by Colmer and Voesenek, (2009) showed that a serious decline of avocado in South California, had been associated with excess water and variously termed to be water injury melarnorhiza, asphyliation or apoplexy (Zentmeyer, 1983). This was actually found

to be a root disease caused by *P. cinnamomi*. Zentmeyer (1983) found that 2-3 years old trees were not sensitive to flooding as plants grown in soils in absence of *P. cinnamomi* could be subjected to up to 9 days of continuous flooding with no apparent ill effects. However, if the soil was infested by the pathogen then flooding of about two days could result into severe root rot. Another study on three species of *P. cinnamomi* showed they was trunk canker on coast live oak and cork oak trees in California (Randy *et al*., 2001) where they had been planted in areas of poorly drained soils subjected to periods of prolonged water saturation **(**Gil *et al.*, 2007).

Historically the occurrence of *Phytophthora* root rot in flooded soils has been attributed to requirements of the pathogen for high soil moisture (Gil *et al.,* 2007). Evidence exists which indicates that soil-water status can exert a determining influence on several epidemiologically important stages in the life of *P.cinnamomi* (Pezeshki, 2001). Some species require a drained soil with matric potentials less than -20 millibars for optimum formation while others require flooded soils of matric potential of 0 millibars (Gil *et al.*, 2007). If the specific water requirements are not satisfied, sporangium formation is significantly reduced (Robin *et al.,* 2001). Once sporangia have formed, their ability to germinate indirectly by release of zoospores also has exacting water requirements. In experiment with *P. megasperma* and *P. cryptogeal* optimum release of zoospores occurred in fully water saturated soils and even a slight drainage of soil to matric potential of -5 or -10 millibars caused significant reduction in the number of zoospores released **(**Smith *et al.*, 2011**)**. Therefore, the ability of zoospore to swim through soil and infect plant roots is dependent upon the availability of large completely water-filled pores in soils that have maximum saturation of water (Kong *et al.,* 2003**).**

Mittler (2006**)** found that host factors associated with oxygen deficiency can also play a significant role in disease development**.** Oxygen quickly becomes deficient in flooded soils when pores are filled with water rather than air, although the degree of anaerobiosis which develops is

mediated by drainage properties of the soil, distribution and continuity of soil pores and respiration of roots and microorganisms (Pezeshki, 2001**)**. Such stress predisposes plants to infections of *Phytophthora cinnamomi*. Although oxygen deficiency could increase disease severity, scientists considered it to be only a secondary contributing factor which prevented regeneration of roots decayed by *Phytophthora cinnamomi* and thus made plants less able to tolerate the effects of chronic root rot (Müller *et al*., 2001). Certainly, the sensitivity of plant roots to periodic flooding could vary greatly depending upon species and season and this could be a determining factor in predisposition (Van Rooyen, 2011). Clearly, the conditions which exist in the flooded soils do not only favour pathogen activity but in some cases can predispose roots to severe infection (Hardham, 2005).

2.12 Management to control root rot

Control of *P. cinnamomi* is difficult because of the pathogen's wide host range and ability to survive in symptomless or tolerant plants **(**Kong *et al*., 2003**)**. Symptomless plants are a major source of spread to previously clean areas, which is a major problem for field nurseries. Preventive measures through sanitation are critical. The best field management practice is to prevent the introduction of the organism into the field through the use of clean seed and clean rootstock as well as utilizing well-drained sandy soils with a low pH (Gil *et al*., 2007). Both sporangium and zoospore production are inhibited at a pH of 3.3. However, at a higher pH of 4.0, sporangium production is still inhibited while zoospore production is not (Müller *et al*., 2001). In container-grown plants, a low pH is not feasible because plant growth is limited. However, for cutting propagation, maintaining mist irrigation of pH between 3.5 and 4.0 will control sporangium formation and thus prevent zoospore formation and disease. Control in container-grown plants can be achieved by using sterile potting soil, chemicals (Fosetyl Al, metalaxyl, or etridiazol), clean rootstock, and coarse, sloping gravel beds on which to place pots **(**Nicolas *et al.*, 2005). Disease severity can be reduced in planted nurseries by planting in raised

beds. Raised beds prevent free water from contacting the plant roots and promoting rapid drainage (Coyier and Robiero, 1996). Pre-planting fumigation may be effective, but it does not reach chlamydospores that may be present in the deeper soils (Coyier and Robiero, 1996).

2.13 Effects of *Phytopthora cinnamomi* and flooding on chlorophyll content

Reeksting *et al*. (2014) found out that in America avocado`s total chlorophyll decreases under *Phytopthora cinnamomi* inoculation. This decrease in chlorophyll due to *Phytopthora cinnamomi* was related to chlorophyll photo-bleaching. These decreases in chlorophyll reflect a reduction in the chlorophyll antenna size of the photosystems from photo-inhibition by reducing energy delivery to the reaction centres (Kate and Giles, 2000).

In most plants, chlorophyll antenna size is an adaptive strategy to reduce light absorption and avoid damage of the photo systems due to *Phytopthora cinnamomi* (Mmayi 2015).This was expected to occur in avocado varieties under study. In most of the varieties at the beginning of exposure to *Phytopthora cinnamomi* inoculums, chlorophyll will reduce but after 48h of treatment the chlorophyll antenna becomes similar to the controls (Reeksting *et al.*, 2014).These varieties have favourable root growth to support faster acclimatization ofphotosynthetic apparatus to *Phytopthora cinnamomi* stress by increasing water absorption and nutrient uptake (Steward *et al*., 2012). This has also been reported in evergreen species under *Phytopthora cinnamomi* stress (Sayed, 2003).

2.14 Effects of waterlogging and *Phytophthora cinnamomi* to chlorophyll fluorescence

Light energy absorbed by chlorophyll molecules in a leaf can be used to drive photosynthesis (photochemistry), excessive energy will be dissipated as heat or it can be re-emitted as light-chlorophyll fluorescence (Liu *et al*., 2012). Any increase in the efficiency of one of the three will result in a decrease in the yield of the other two (Maxwell and Johnson, 2000). It is well known that photoinhibition is one of the primary physiological consequences of water-

logging and that alteration of PSII activity under water stress are related to photoinhibition rather than to a direct damage to PSII (Efeoglu, 2009).

Measuring chlorophyll fluorescence has become a very useful technique in obtaining rapid qualitative and quantitative information on photosynthesis (Rohácek and Bartak, 1999), and it can provide information on the relationship between structure and function of photosystem II (PSII) reaction centre (Stewards *et al*., 2012). Chlorophyll fluorescence analysis is a useful, non-invasive, powerful, and reliable technique to assess the changes in function of PSII under different environments (Liu *et al*., 2012; Mauchamp and Methy, 2004). It can check the composition and organization of photosystems (Jiang *et al*., 2008), the excitation energy transfer, the photochemistry, and the effects of various stresses on plants (Liu *et al*., 2012). Chlorophyll fluorescence provides useful information about leaf photosynthetic performance of many plants under drought stress (Liu *et al*., 2012). Furthermore, chlorophyll fluorescence can tell the extent to which PSII is using the energy absorbed by chlorophyll (Zhou *et al*., 2011), and the extent to which it is being damaged by excessive light (Maxwell and Johnson, 2000). Waterlogged avocado plants that were infected were expected to show a reduction of the photochemical chlorophyll fluorescence quenching, PSII quantum yield and electron transport rate and more heat dissipation (Dias and Bruggemann, 2010).

Under high irradiance, however, the PSII reaction centres will absorb excessive light energy which eventually results in the impairment or inactivation of the chlorophyll-containing reaction centres of the chloroplasts (Bertaminia *et al*., 2006). When studying maize genotypes, Liu *et al*. (2012) analysed chlorophyll fluorescence that showed that photosystem (PSII) was rather tolerant to the water stress imposed. According to them, water stress caused a slight decrease in the efficiency of excitation capture by open PSII reaction centre. Declining values of Fv/Fm are an indicator of stress (Liu *et al*., 2012). Dark adapted values of Fv/Fm reflect the potential

quantum efficiency of PSII and have previously been used as sensitive indicators of plant photosynthetic performance, with optimal values for healthy plants generally being 0.83 (Burke, 2007; Liu *et al*., 2012). Values are lower when the plants are exposed to stress, indicating the phenomenon of photo-inhibition or the degree of damage to PSII complex (Kate and Giles, 2000).

Fv/Fm was used to screen maize (Liu *et al*., 2012) and was found to be correlated with decreased CO_2 assimilation and electron transport (Sayed, 2003). Decline in Fv/Fm in avocado genotypes when water-logged, suggested that photo-inhibition is accompanied by an over-reduction of PSII (Reeksting *et al*., 2014). This study just like that of Colom and Vazzana (2003) hen studying maize showed that with increasing irradiance, there was a steady decline in qP,ϕPSII, open PSII energy capture efficiency (Fv/Fm) and a clear increase in non-photochemical quenching (NPQ). It is however not clear how these parameters are affected by water logging and inoculation by *P. cinnamon* on avocado. The qP is an indication of the proportion of open PSII reaction centres, and translates light quantum energy into chemical energy process, which reflects the photosynthetic efficiency and the light use situation of plant (Fracheboud *et al*., 1999). NPQ can represent the energy which cannot be utilized to transport photosynthetic electrons but be dissipated harmlessly as heat energy from PSII antennae (Kate and Giles, 2000) and (Fracheboud *et al*., 1999). In avocado rootstocks, a decrease of the qP was observed in response to the *P. cinnamomi* treatment, indicating that a larger percentage of the PSII reaction centres would close at any time (Reekstings *et al*., 2014), which also indicated that the balance between excitation rate and electron transfer rate have changed (Efeoğlu, 2009). It is therefore interesting to establish the trend of these effects in avocado rootstocks under water logging. Fv/Fm is of great value in assessing the relative contributions of PSII photochemical capacity and thermal decay processes to the overall efficiency of photochemistry at PSII in avocado plants (Liu *et al*., 2012).

ϕPSII is the effective quantum yield of photochemical energy conversion in PSII (Ronácek and Bartak, 1999). ϕPSII increase is related to significant reductions of Fv/Fm (Colom, 2003). Such reductions occur with increase in thermal energy of dissipation indicated by NPQ. Increase in non-photochemical fluorescence quenching, as one means of estimating the level of energy dissipation, is expected to have increase incident photon flux densities at waterlogging and *P. cinnamomi* in avocado leaves. The decrease of qP and ϕPSII is also expected under this stress (Shangguan *et al*., 2000).

Xanthophyll cycle relying on photo-protection is believed to be the main mechanism for plants to deal with excessive light energy (Liu *et al*., 2012), and it plays an indirect role in thermal dissipation by mediating a critical conformational change within the PSII antenna (Ort, 2001). With the increase of NPQ of xanthophyll cycle, excessive energy was dissipated as thermal energy to protect the maize leaf from light-induced damage in draught.

The variation trend of NPQ increased along with the increasing irradiation. Ort (2001) indicated that the NPQ got involved in the competition between the thermal dissipation of chlorophyll *a* and fluorescence emission as well as photosynthesis.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area and Experimental Materials

Avocado fruit varieties were collected from the following parts of Kenya; Maseno division, Nyando division, Kisumu East division (Kisumu county) and Busia-Budalangi division (Busia county), and were taken to Museums of Kenya Herbarium for confirmation and identification.

Avocado fruits were kept to ripen then seeds extracted and planted at Maseno Botanical Garden in polythene bags. A total of four rootstocks commonly grown in these regions were planted, namely; Fuerte, Booth 7, Puebla and Pinkerton. The varieties were identified with the following characteristics according to Reeksting *et al*. (2014): Fuerte fruits were pear with flat area on bottom corner, green colour ripe fruit, thin skin, smooth fruit surface and fruits mature early. Booth7 were spheroid oborte, fruit apex rounded, small in size, bright green fruit that are slightly pebbed, Glossy, thick and woody skin. Puebla fruits were small in size, onyx black skin that is thin and smooth, very greamy and succulent flesh. Pinkerton had fruits that are pear shaped with well developed long neck, course dark green ripe fruits and medium thick skin that are mid in maturing.

Mwai (2001) classified Maseno to be located at Latitude extent 0^0 1`N – $0^0$ 12`S and Longitude extent 34^0 25`E – $34^0$47`E, Maseno is at approximate 1500m above sea level, soils in Maseno are acrisol deep reddish brown clay and well drained and that Maseno receives rains averaging to 1750 mm per annum with a mean temperature of 28.7^0C. The seedlings were then transferred to a greenhouse in KALRO (Kibos) research centre after three months. Greenhouse growth conditions were 25°C-40°C/20°C-30°C (day/night) temperature, 14/10-h (light/dark) photoperiod, and 64-77% relative humidity. Flooding and inoculation treatments were then

induced after three weeks of acclimation. According to Japheth *et al.* (2014), Kibos is located at 34^0 48`E, $0^0$ 04`N and1144m above sea level with clay loamy soil and long term mean annual rainfall of 1440mm.It`s temperatures range from 15.3 ^{0}C to 30 ^{0}C.

3.2. Isolation, culturing and identification of *Phytophthora cinnamomi*

3.2.1. Isolation

Soil samples of 5kg were collected from each site where avocado rootstocks had been picked. Baiting technique of isolation was used to get the pathogen. Uninfected ripe avocado fruit was used due to its susceptibility to the pathogen (Pandey *et al*., 2010). A ripe avocado fruit was thoroughly washed by tap water and then placed in a basin containing fresh flooded soils with 1l litre distilled water and incubated in the dark for 72hrs. After baiting the baits were removed, blotted on a paper towel and plated on PARPH-V8 selective agar. PARPH-V8 selective agar composed of 20g agar, 200ml filtered v8 broth, 800ml deionised water, 50g hymexazol, 5mg pirimicin, 10mg rifampicin, 250mg ampicilin and 125mg a.i pentachloronitrobenzene.

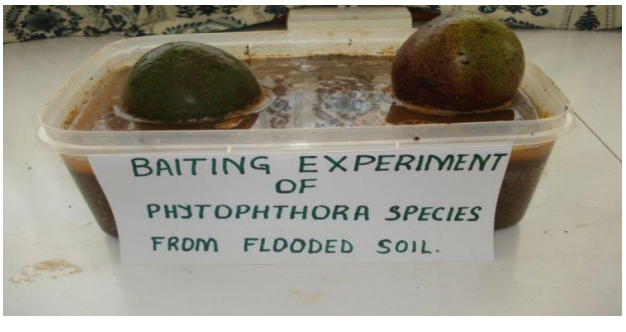

Plate 3.2.1.: Baiting using uninfected avocado fruit

V8 agar was prepared using vegetables according to the method of Pegg *et al*. (2002), where; 3 large tomatoes, 3 stalks of celery, 5 carrots, 1 small beet, 1/4 head of fresh cabbage, 1-2 bell pepper, 2 cups spinach, 3 kale leaves, 1/4 sweet onion and 1/4 clove garlic were cut, mixed

and placed in 800JEXL Breville juice fountain elite 10000-watt juice extractor. The juice was then sieved and kept in the refrigerator to remain fresh.

3.2.2. Culturing

Infected regions with brown colorations were cut out from baits and cultured on a PDA media, placed in an incubator at temperature of 24 ^{0}C, then observed after 3 to 4 days.

3.2.3. Identification

Staining was done using lactophenyl cotton blue, and then was observed under a microscope under a magnification of x10. Identification of the fungus was done according to Erwin *et al*. (1996). According to Erwin *et al*. (1996) Sporangia should be ovoid, obpyriform with an apical thickening, tapered or rounded at the base, and terminally borne forming a rosette like shape.

3.3. Screening avocado rootstocks for disease resistance

3.3.1. Flooding and Inoculation Treatments

Flooding treatments were introduced when seedlings were three months old and this was after two weeks of transplanting of avocado rootstock seedlings (plate 3.3.1). Inoculations were done at 7 days intervals thereafter. The avocado rootstocks seedlings planted in 10 litre pots were immersed in 20 litre plastic pots containing distilled water almost half full. Two control treatments involved avocado seedlings grown in 10 litre plastic pots, and daily provided with water and another one where the avocado seedlings were planted into 10 litre plastic pots and immersed into 20 litres plastic pots containing water half full but were not inoculated.

The treatments were as indicated below and were replicated three times.

$T_{1;}$ - Un-flooded-un-inoculated (Control)

T2;-Unflooded-inoculated

T3;-Flooded uninoculated

$T_{4;}$ - Flooded-inoculated

The pots containing the avocado rootstock seedlings were laid out in a greenhouse in a completely randomized design. The inoculums were soaked with dilute vegetable juice broth as described by Wilcox (1989). The test fungus was serially diluted to a concentration of approximately $1x10^7$cfu/ml. It was added to potted plants at a rate of $30cm^3$ per $1000cm^3$ of potting medium.

Plate 3.3.1 Arrangement of avocado rootstocks flooded and inoculated with *P. cinnamomi* in a greenhouse at kibos KARI.

3.3. Determination of plant growth parameters

At the end of two months since the initiation of treatments, plant growth parameters were measured at 80 DAI.

3.4.1. Plant height.

Plant height was determined by measuring the height of the plants from soil surface to the tallest leaf tip in plant using a metre rule. This was done on a specifically chosen plant throughout the experiment.

3.4.2. Plant stem diameter

Plant stem diameter was measured using a vernier calliper at a height of 20 cm from the soil surface in the pot.

3.4.3. Plant fresh weights and dry weights

One plant was harvested in each treatment and then rinsed with tap water to remove the soil particle from the leaves then roots were immersed in a bucket of water to remove soil that adhered to the root surface. The whole plant fresh weight was measured immediately after harvesting by using a weighing balance (Denver instrument model XL31000). The plants were dried in an oven for at least 72 hours at 70°C to Constant weights for dry weight determination.

3.4.4. Leaf area

Leaf area was determined according to Lal and Subba (1951) whereby the leaf was traced over a graph paper and its area worked out by counting the number of square centimeters and adding the area of partial squares.

3.5. Determination of disease incidence caused by necrosis and lesions

The disease incidence was determined according to Corcobado *et al.* (2013), rated on the basis of root necrosis and lesions. A visual estimate of the percentage of rotting roots system was done. Due to different genetic variations in size and vigour of rootstocks, pathogen flooding regime was expressed and analyzed as percentage of the mean weight of the un-inoculated plants for the same rootstocks flooding duration combination. Similarly, to compare effects of periodic water logging events in the absence of *Phytophthora spp,* root and shoot weight of individuals that were un-inoculated were determined. Plants in each flooding treatments were expressed as a percentage of the mean weight of the un-flooded control plants of the same rootstocks.

The lesion incidences were recorded with the following scores according to Jeffers and Sisco (2009);

0 *- no infection,*

1 *- Low infection,*

2 *- Moderate infection,*

3 *- Severe infection,*

4 *- Completely infected*

3.6. Determination of chlorophyll concentration

Chlorophyll concentration was determined according to Netondo (1999). The third youngest leaf was sampled for all treatments. In the laboratory 0.5g of the fresh leaf tissue was weighed and cut into small pieces into specimen bottle. Ten millilitres of 80% acetone was added and the set kept in the dark for 4 days at room temperatures for the chlorophyll to be extracted by the acetone. Absorbance of the chlorophyll of the solution measured using a spectrophotometer (Nova spec II, Pharmacia Biotech, Cambridge, England) at 645 and 663nm to determine the

chlorophyll *a*and *b* content. The respective chlorophyll concentration in mg of chlorophyll per gram of the leaf collected was calculated using the formula of Arnon (1949) as follows: -

Chl a = 12.7(D663) - 2.67(D645) × V/1000 × W [mg *Chl a* g^{-1}leaf tissue];

Chl b = 22.9(D645) -4.68(D663) × V/1000 × W [mg *Chl b* g^{-1}leaf tissue];

Total chlorophyll concentration was calculated as *chla +chlb*.

Where:

Chl a is chlorophyll a concentrations; *chl b* is chlorophyll b concentrations; D = absorbance measured at wavelengths 645nm and 663nm; V= volume in ml of acetone extract used and W= fresh weight (g) of leaf from which the extract was made.

3.7. Determination of chlorophyll fluorescence parameters

Chlorophyll fluorescence measurements were carried out using a portable fluorescence monitoring system (Hansatech model FMS 2; Hansatech Instruments, England) on the first fully opened and exposed leaf at an interval of two weeks. Leaves were dark-adapted for 15 minutes, using the dark adaptation clips and then illuminated for six seconds to induce fluorescence. The leaves were continuously illuminated with a white actinic light (200 µmol m^{-2} s^{-1}). The initial fluorescence (Fo) and the maximum fluorescence (Fm) was measured, and the variable fluorescence (Fv = Fm - Fo) and the Fv/Fo ratio calculated. The potential minimum efficiency of PSII (Fv/Fm) of dark-adapted leaves was calculated as Fv/Fm = (Fm-Fo)/Fm. The parameters of fast chlorophyll fluorescence, maximum fluorescence yield from PSII following a saturating pulse of photons in a light-adapted plant (Fm'), steady state yield of PSII fluorescence in the light (Fs), and electron transport rate through PSII (ETR) was determined during the day between 11:00 am and 1:00 pm according to Maricle *et al*. (2007).

3.8. Statistical data analysis

The data collected from this study were subjected to analysis of variance (ANOVA) using SAS statistical computer package (Steel *et al*., 2006). Fisher's LSD test at 5% level was used to separate the treatment means.

CHAPTER FOUR

RESULTS

4.1. Morphological identification of *Phytopthora cinnamomi*

After staining the hyphae with lactophenol cotton blue, sporangia observed were ovoid, obpyriform with an apical thickening, tapered or rounded at the base, and terminally borne forming a rosette like shape as observed by microscope. Sporangia, which release motile zoospores (plate 4.1a). Chlamydospores were produced abundantly axenically and from infected tissue. They were borne from hyphae, and globose with thinner walls (plate 4.1b). Sizes ranged from 31 to 50 µm in diameter and were either terminal to intercalary in the mycelium. The fungus was heterothallic, requiring compatible types to sporulate sexually. Antheridia were amphigynous, averaging 19x17 µm. Oogonia were round with a tapered base, smooth, hyaline to yellow, with size ranging from 21 to 58 µm. Oospores were hyaline to yellow, and plerotic. Sizes range from 19 to 54 µm. Petri dishes in plate 4.1a and 4.1b shows morphologies of colonies of isolates of *Phytopthora cinnamomi* plate growing on vegetable agar medium.

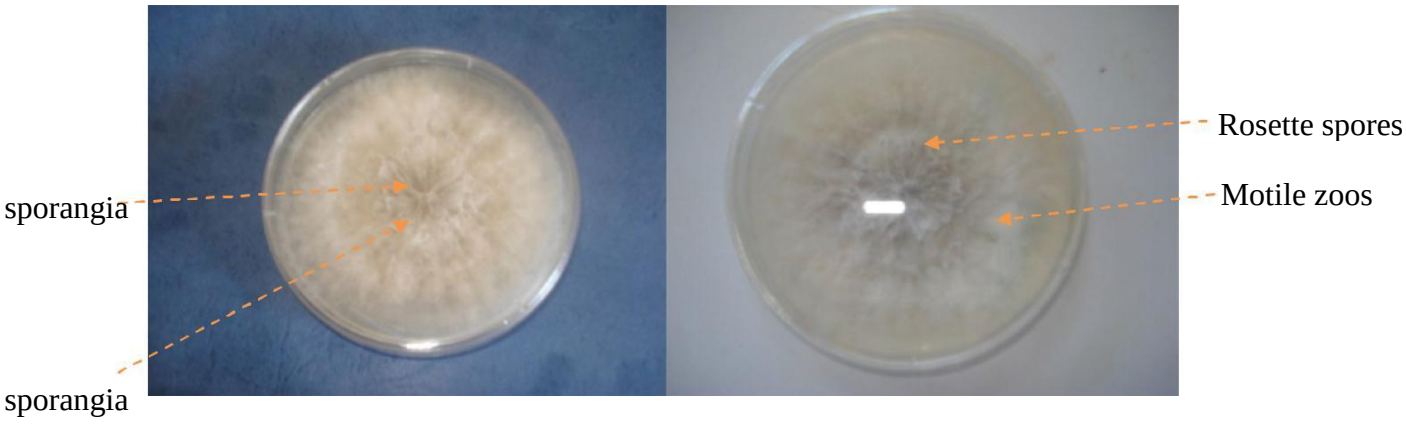

Plate 4.1a : Rosette like shape sporangia indicated by the arrows

Plate 4.1b : Motile zoos released from Rosette indicated by the arrows

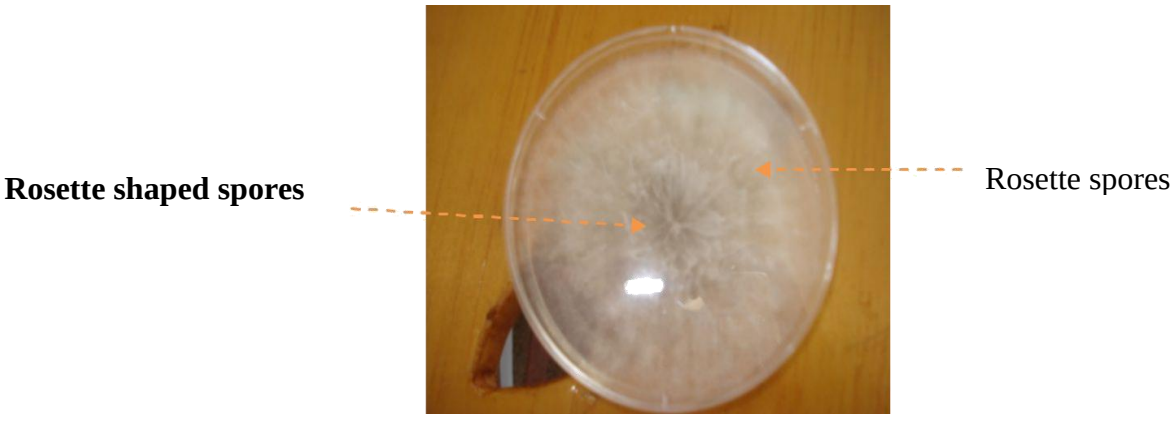

Plate 4.1c: Showing morphological characteristics of the *P. cinnamomi* pathogen indicated by the arrows grown on PDA media

4.2. Plant growth

4.2.1. Plant height

Fuerte rootstocks had generally the highest plant height compared to the other avocado rootstocks. Plant height was highly significantly different in Fuerte rootstocks compared to other rootstocks except for the flooded and inoculated treatments (Table 4.2.1). There were significant interactions in plant height ($p \leq 0.05$) between treatments and rootstocks (Appendix 1). Plants under control treatment had a significantly highest plant height when compared to the avocado rootstocks under other treatments (Table 4.2.1).

Table 4.2.1: Plant height (cm) of avocado rootstocks: control (Un-flooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Plant height (cm)				
	Fuerte	Booth 7	Puebla	Pinkerton	**Treatments mean**
Treatments					
Control	94.3a	55.4b	47.8c	0.458c	**60.8a**
Unflooded-Inoculated	55.6a	46.3b	43.6b	0.445b	**47.4b**
Flooded and Uninoculated	57.2a	39.0b	41.3b	0.432b	**45.2c**
Flooded and inoculated	40.8a	36.2c	38.6b	0.429a	**39.6d**
Rootstocks mean	**61.9a**	**44.2b**	**42.8b**	**44.1c**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. differences shown across for unbold values.

4.2.2. Stem diameter

Fuerte rootstocks had significantly ($p \leq 0.05$) the largest stem diameter compared to Booth 7, Puebla and Pinkerton rootstocks (Table 4.2.2). Stem diameter was significantly different between Fuerte and other rootstocks, except for the unflooded-inoculated treatment. There were significant interactions in stem diameter between rootstocks and treatments (Appendix 1). There were no significant differences in stem diameter among the four treatments.

Table 4.2.2; Mean stem diameter (cm) of avocado rootstocks: control (Un-flooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Plant stem diameter (cm)				
	Fuerte	Booth 7	Puebla	Pinkerton	**Treatments mean**
Treatments					
Control	33.8a	31.4b	29.9c	28.6c	**31.0a**
Unflooded-inoculated	34.3a	31.9a	30.4a	29.1a	**31.4a**
Flooded and Uninoculated	33.3a	30.9b	29.4b	28.1c	**30.5a**
Flooded and inoculated	32.7a	30.3b	28.8b	28.0c	**29.8a**
Rootstocks mean	**33.5a**	**31.1b**	**29.6b**	**28.3c**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. Differences shown across for unbold values.

4.2.3. Plant fresh weights

In Table 4.2.3, Plant fresh weights decreased significantly ($p \geq 0.05$) under uninoculated-flooded and flooded inoculated treatments (Table 4.2.3). Fuerte rootstocks had the largest mean plant fresh weight value. There were significant interactions between rootstocks and treatments in plant fresh weights (Appendix 1). Mean of control plants was slightly higher compared to the mean of plant fresh weight of other treatments; Infected Flooded –inoculated, and Flooded – uninoculated. Means of rootstock Fuerte and Booth 7 were highly significantly different when compared to Pinkerton and Puebla.

4.2.4. Plant Dry weights

In Table 4.2.4, the dry weight of Fuerte rootstocks was significantly reduced at flooded uninoculated and flooded inoculated treatments (Table 4.2.4). There were significant differences

($p \leq 0.05$) in plant total dry weight among the treatments and rootstocks. There were significant interactions in dry weights between treatments and rootstocks (Appendix 1). Mean dry weight of Fuerte rootstock (8.61) was highly significant different when compared to the mean of Booth 7, Pinkerton and Puebla rootstocks.

4.2.5. Leaf area

Leaf area significantly reduced under flooding and inoculated treatments (Table 4.2.5), especially in Puebla, Pinkerton and Booth7 rootstocks. There were significant differences in leaf area among treatments and rootstocks. There were no significant interactions in leaf area between treatments and rootstocks (Appendix 1).

Table 4.2.3; Mean fresh weights (g) of avocado rootstocks: control (Un-flooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Plant fresh weights (mg)				
	Fuerte	Booth 7	Puebla	Pinkerton	**Treatments mean**
Treatments					
Control	15.864a	13.940a	13.300a	13.831a	**14.234a**
Unflooded-noculated	11.822a	9.898b	9.789b	9.258b	**10.191b**
Flooded and Uninoculated	7.896a	5.972b	5.332b	5.863b	**7.016c**
Flooded and inoculated	0.326a	0.317a	0.286b	0.296b	**7.016c**
Rootstock means	**11.057a**	**9.133a**	**8.493b**	**9.024b**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. Differences shown a cross for unbold values.

Table 4.2.4; Mean total dry weight (g) of avocado rootstocks: control (Un-flooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Plant total dry weight (mg)				
	Fuerte	Booth 7	Puebla	Pinkerton	**Treatments means**
Treatments					
Control	10.654a	8.709a	7.995a	8.595a	**8.988a**
Unflooded-Inoculated	9.353a	7.408b	6.694b	7.294b	**7.687a**
Flooded and Uninoculated	6.884a	4.939b	4.225b	4.825b	**5.873b**
Flooded and inoculated	8.819a	9.223a	4.976b	5.583b	**5.873b**
Rootstock means	**8.607a**	**6.662b**	**5.948b**	**6.548b**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. Differences shown a cross for unbold values.

Table 4.2.5; Mean leaf area (cm^2) of avocado rootstocks: control (Un-flooded-un-inoculated), unflooded-inoculated, flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Leaf area in cm^2				
	Fuerte	Booth 7	Puebla	Pinkerton	**Treatments mean**
Treatments					
Control	12.369a	10.670a	11.349b	10.729b	**11.279a**
Unflooded-Inoculated	10.661a	8.962a	9.641a	9.021a	**9.572b**
Flooded and Uninoculated	11.653a	9.954a	10.633a	11.013a	**10.563a**
Flooded and Inoculated	9.895a	12.020a	11.170b	8.339c	**10.172b**
Rootstock means	**11.486a**	**9.787b**	**10.467b**	**9.846b**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. Differences shown across for unbold values.

4.2.6. Disease incidence

Control avocado plants had no Lesions, more lesions were found in Fuerte rootstocks. Pinkerton lacked lesions under Uninoculated and flooded treatments and when flooded and inoculated treatments. Fuerte, Booth7 and Puebla had more lesions under inoculated, and uninoculated flooded treatment but when flooded and inoculated Fuerte and Booth7 were much affected (Table 4.2.6).Necrosis and lesions as a parameter showed significant differences ($p \leq 0.05$) among treatments and rootstocks and also for the interaction between treatments and rootstocks (Appendix 2). Mean of disease incidences at control showed significant differences when it was compared to any other treatment i.e. under control, Flooded –uninoculated, Flooded –inoculated, and Inoculated as in (Appendix 2). Mean of rootstocks significantly differed whenever each was compared to the other as; Fuerte, Puebla, Pinkerton and Booth 7.

Table 4.2.6; Mean disease incidences in avocado rootstocks subjected to various treatments i.e.control (Unflooded-un-inoculated), Unflooded- inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI.

	Fuerte	Booth 7	Necrosis Puebla	Pinkerton	**Treatments means**
Treatments					
Control	0.0a	0.0a	0.0a	0.0a	**0.000d**
Unflooded Inoculated	2.667a	1.000b	1.000b	1.00b	**1.500b**
Flooded and Uninoculated	1.667a	1.00a	1.00a	0.00b	**0.833c**
Flooded and inoculated	4.000a	4.000a	0.00b	0.00b	**2.083a**
Rootstocks means	**2.100a**	**1.446b**	**1.447c**	**1.447d**	

Values are means of three replicates. Means with the same letter for rootstocks are not significantly different. Note; sig. Differences shown across for unbold values.

4.3. Chlorophyll concentration

4.3.1. Chlorophyll *a*

Chlorophyll *a* was significantly ($p \leq 0.05$) decreased by flooding and *phytophthora cinnamon* inoculation. Fuerte and Booth 7 rootstocks were much affected (Figure 4.3.1). There were significant interactions in Chlorophyll *a* concentrations between treatments and rootstocks (Appendix 2). Chlorophyll a content values were as follows; control (26.75), Flooded –uninoculated (17.24), Flooded – inoculated (10.05), and Inoculated (20.42) (Appendix 2). Mean of variety Pinkerton (21.31) and Puebla (22.15) were significantly different when each was compared to each of the following varieties; Fuerte (15.33) and Booth 7 (15.56).

4.3.2. Chlorophyll *b*

There were significant ($p \leq 0.05$) decreases in chlorophyll *b* concentration in Fuerte and Booth7 rootstocks under Flooded and inoculated treatments (Figure 4.3.2). Significant differences in chlorophyll *b* concentration occurred among treatments and rootstocks. There were significant interactions between treatments and rootstocks (Appendix 2). Mean of Pinkerton rootstock (22.97) and Puebla (22.040) were significantly different when compared to other avocado rootstocks, i.e Fuerte (15.840) and Booth 7 (16.112).

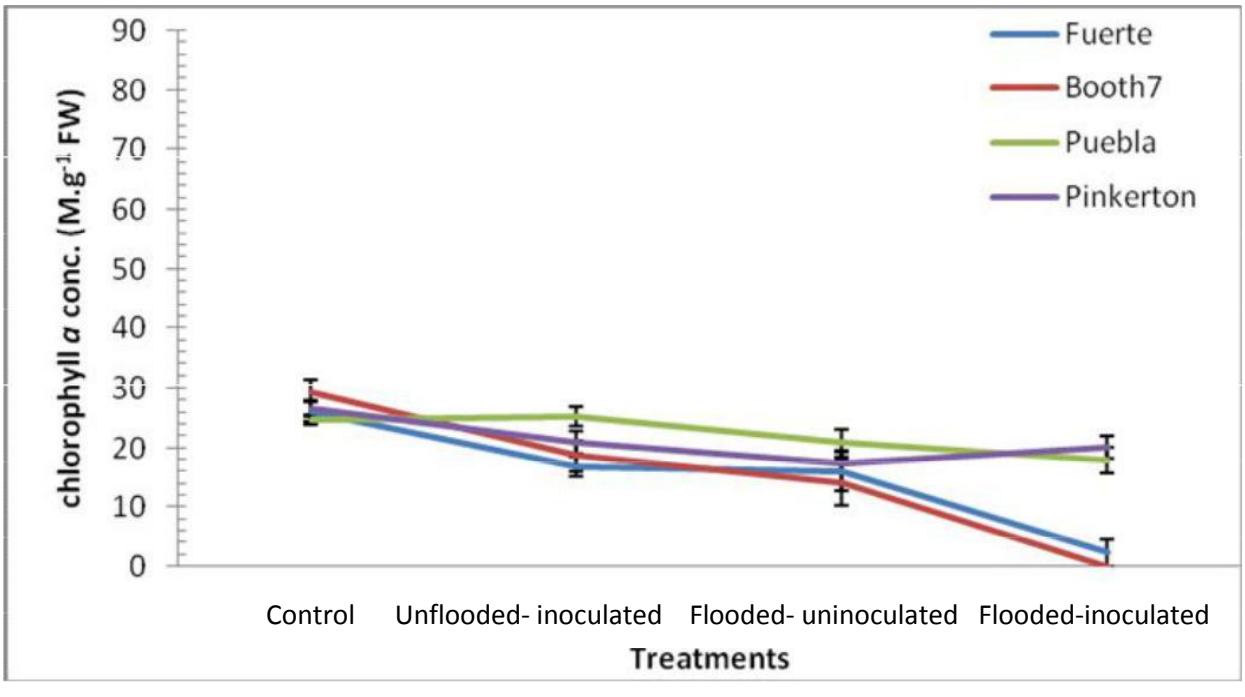

Fig. 4.3.1. Chlorophyll *a* conc. (m.g^{-1} FW) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

4.3.3. Total chlorophyll (mg.g^{-1})

Total chlorophyll concentration significantly ($p \leq 0.05$) decreased in Fuerte and Booth 7 under flooded–uninoculated and flooded-inoculated conditions (Figure 4.3.3). Pinkerton Rootstocks were less affected under Flooded and inoculation treatment. There were significant differences in total chlorophyll content among treatments and rootstocks. The interactions between treatments and rootstocks were also significant (Appendix 2).

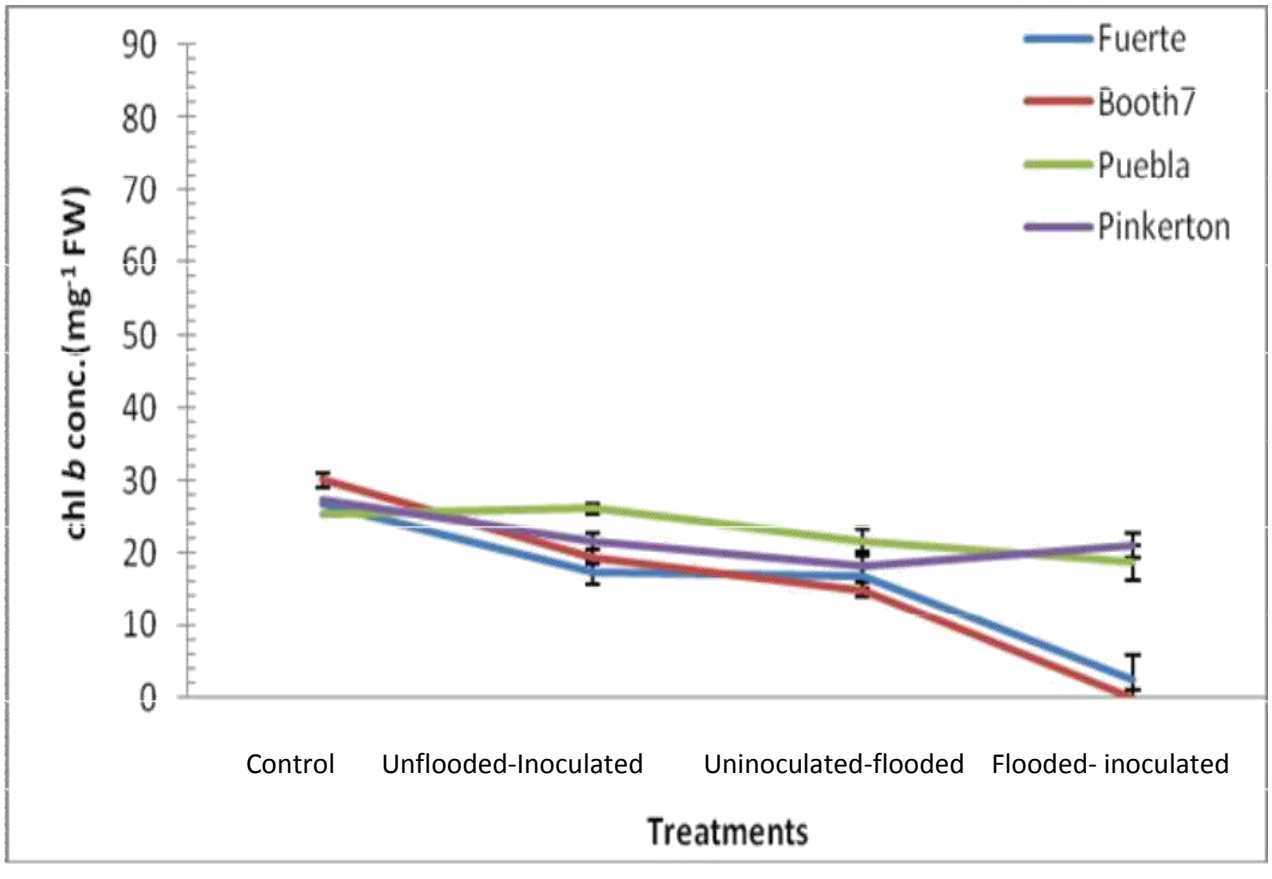

Fig. 4.3.2.Chlorophyll *b* conc. (m.g^{-1} FW) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

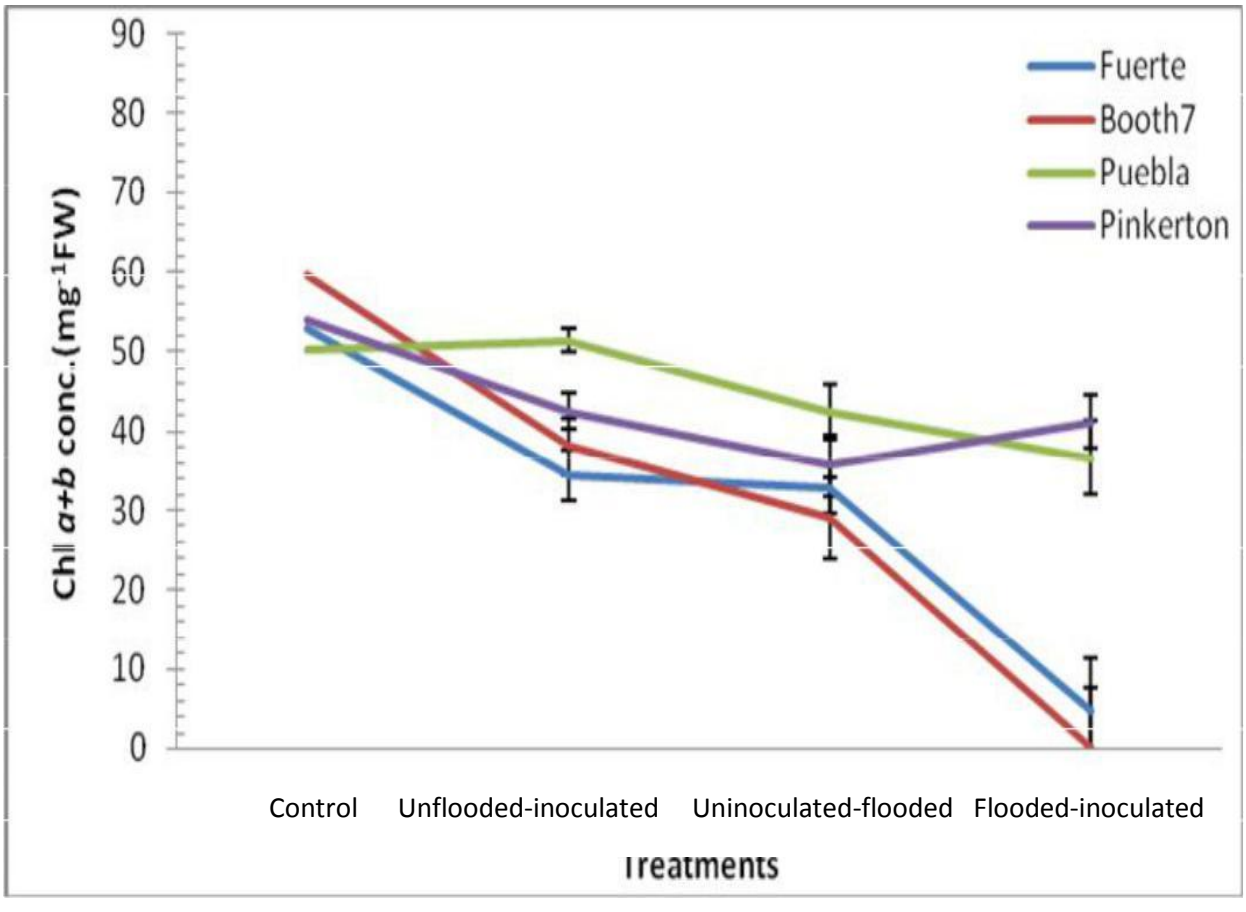

Fig. 4.3.3.Chlorophyll *a*+*b* conc. (m.g^{-1} FW) of avocado rootstocks: control (Un-flooded-un-inoculated), unflooded-inoculated, flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

4.3.4. Maximum quantum yield (FV/FM)

Pinkerton and Puebla rootstocks were less affected by flooding and inoculation treatments (Figure 4.3.4). There were significant ($p \leq 0.05$) decreases in FV/FM among Fuerte and Booth 7 rootstocks under Flooded-uninoculated and flooded-inoculated treatments. There was a significant interaction between treatments and rootstocks (Appendix 2). Mean of variety Puebla (0.63) was significantly different when compared to each of the following rootstocks; Pinkerton (0.42), Fuerte (0.44) and Booth 7 (0.32).

4.3.4. Maximum quantum yield (FV/FM)

Pinkerton and Puebla rootstocks were less affected by flooding and inoculation treatments (Figure 4.3.4). There were significant ($p \leq 0.05$) decreases in FV/FM among Fuerte and Booth 7 rootstocks under Flooded–uninoculated and flooded-inoculated treatments. There was a significant interaction in FV/FM between treatments and rootstocks (Appendix 2). Mean of Puebla rootstock (0.63) was significantly different when compared to the other rootstocks; Pinkerton (0.42), Fuerte (0.44) and Booth 7 (0.32).

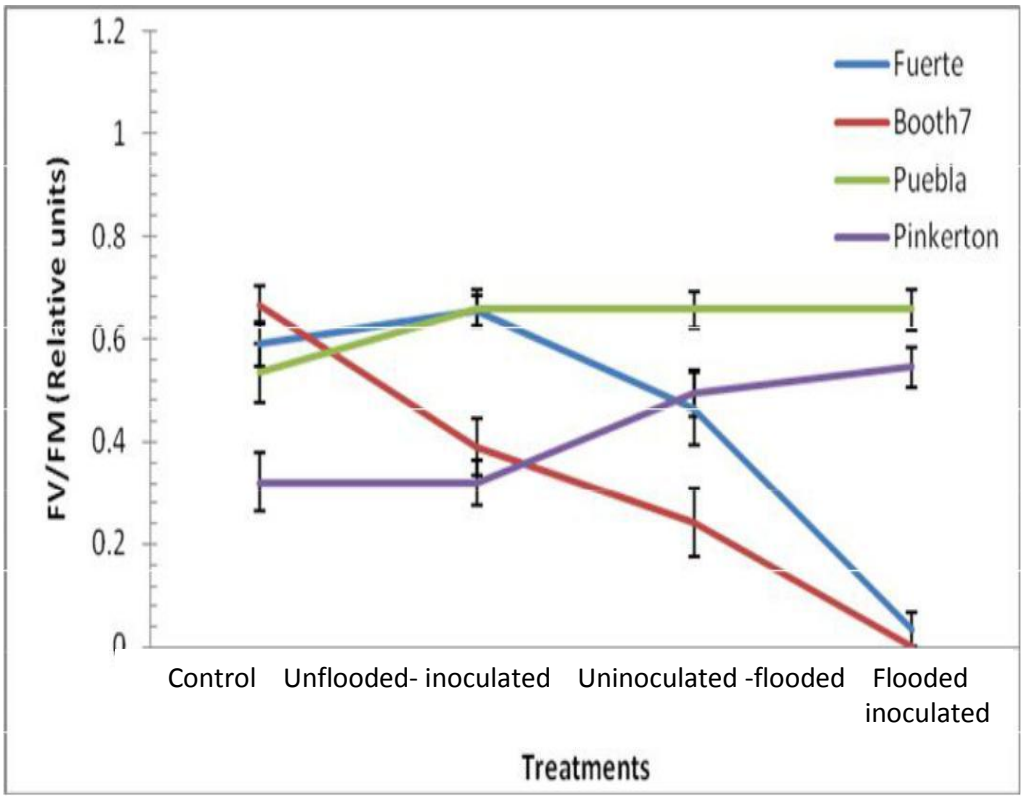

Fig. 4.3.4. FV/FM (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

4.3.5. Effective quantum yield (ϕPSII)

Fuerte and Booth7 rootstocks were much affected by the flooded-uninoculated and flooded - inoculated treatments (Figure 4.3.5). There were significant ($p \leq 0.05$) decreases in ϕPSII among treatments and rootstocks. The interactions between treatments and rootstocks were also significant (Appendix 2). Mean of rootstock Puebla (0.60) was significantly different when compared to each of the following; Pinkerton (0.45), Fuerte (0.42) and Booth 7 (0.30).

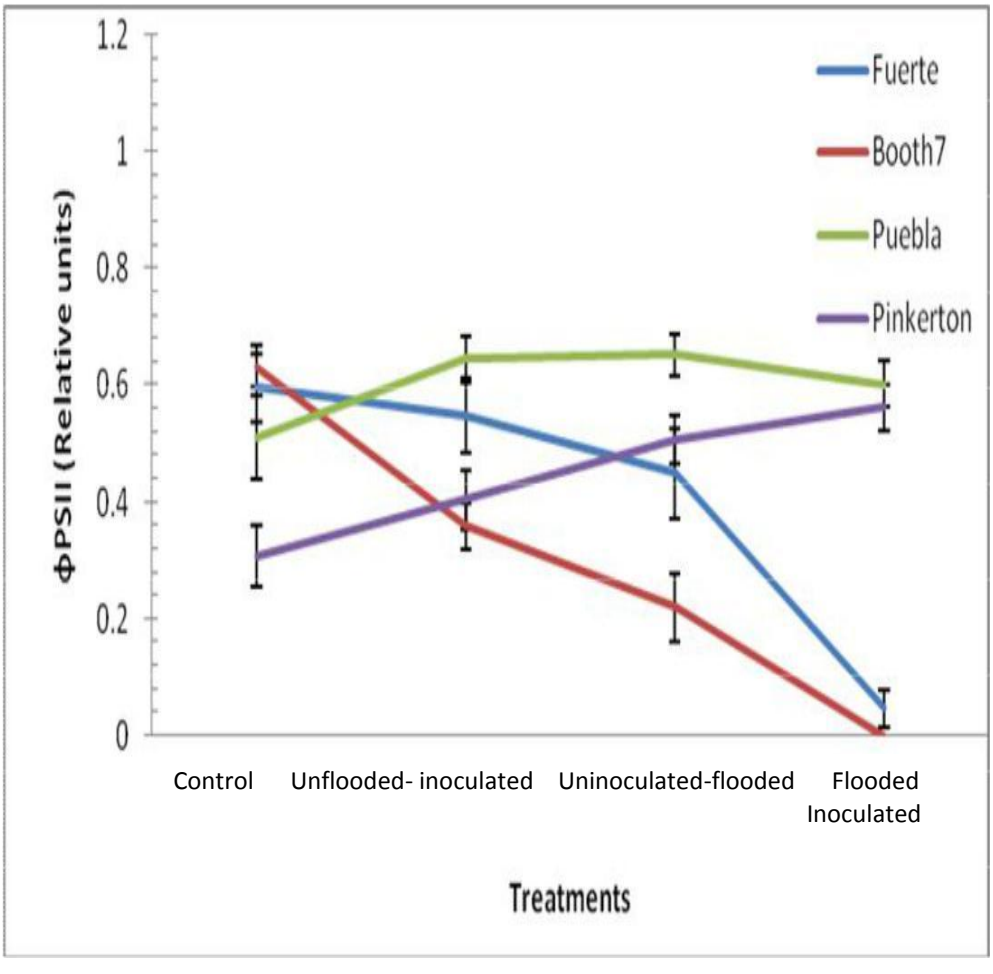

Fig. 4.3.5.ϕPSII (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

4.3.6. Non-photochemical quenching (NPQ)

Non-photochemical quenching decreased significantly ($p \leq 0.05$) in Fuerte and Booth 7 avocado rootstocks under flooded-uninoculated and flooded- inoculated conditions (Figure 4.3.6). On the other hand Puebla and Pinkerton rootstocks experienced some increases in NPQ under similar conditions. There were no significant differences in NPQ between Fuerte and Pinkerton rootstocks under unflooded inoculated and uninoculated flooded. There were no significant interactions between treatments and rootstocks (Appendix 2).

4.3.7. Electron transport rate (ETR)

ETR values were significantly ($p \leq 0.05$) reduced under uninoculated-flooded and flooded-inoculated conditions in both Booth7 and Fuerte rootstocks (Figure 4.3.7). Electron transport rate showed significant decreases ($p \leq 0.05$) among treatments and rootstocks. There was a significant interaction between treatments and rootstocks (Appendix 2).

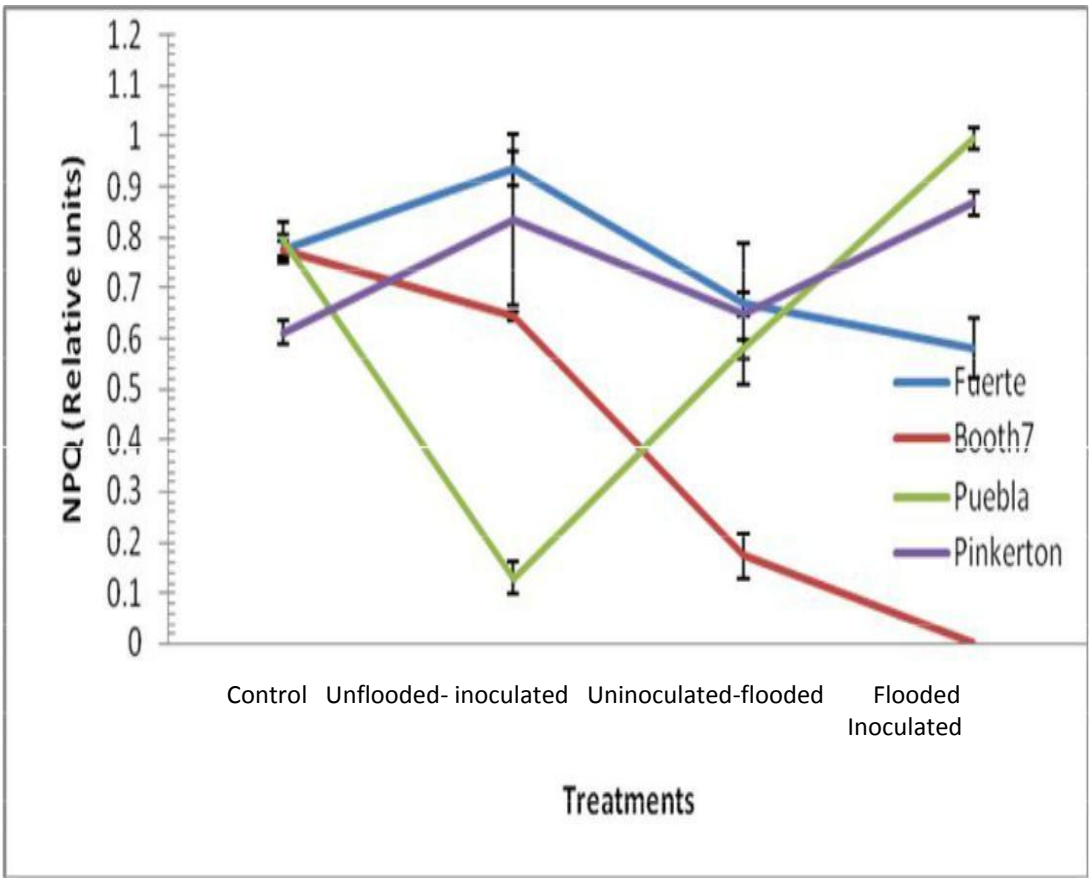

Fig. 4.3.6. NPQ (Relative Units) of avocado rootstocks: control (Unflooded-un-inoculated), Unflooded-inoculated, Flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates ± SE.

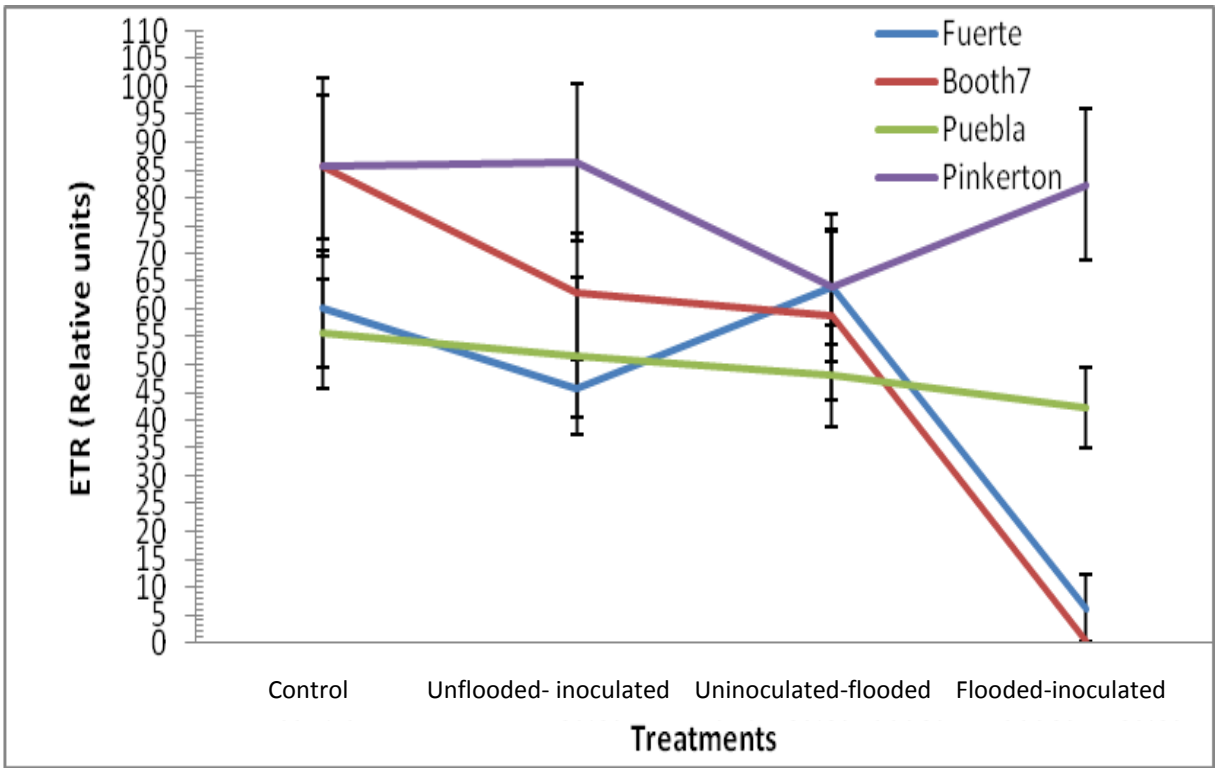

Fig. 4.3.7. ETR (Relative Units) of avocado rootstocks: control (Un-flooded-un-inoculated), unflooded-inoculated, flooded-uninoculated and Flooded-inoculated conditions 80 DAI. Values are means of three replicates±S.E.

CHAPTER FIVE

DISCUSSION

5.1 Morphological identification of *P. cinnamomi*

After baiting and culturing *Phytophthora cinnamomi* was found present in fruits. The soil-borne motile oomycete, *Phytophthora cinnamomi*, caused *Phytophthora* root rot (PRR). *Phytophthora cinnamomi* is motile; this may be the cause of the faster infection which resulted in the feeder roots of avocado plants becoming brittle and turning black, as the root tissue decayed. This restricts water and nutrient uptake by the trees and leads to branch-dieback and eventual tree death (Reekstings *et al*., 2014) as found in Fuerte and Booth7 rootstocks when flooded and inoculated. These findings comparable to other results by Reekstings *et al*. (2014). Chlamydo spores were produced abundantly axenically and from infected fruits tissue. Culturing on PDA media, chlamydophores were seen to be borne from hyphae, and were globose with thinner walls (plate 4.1b). Sizes ranged from 31 to 50 µm in diameter and were either terminal to intercalary in the mycelium. The fungus was heterothallic, requiring compatible types to sporulate sexually. Antheridia were amphigynous, averaging 19x17 µm. Oogonia were round with a tapered base, smooth, hyaline to yellow, with size ranging from 21 to 58 µm. Oospores were hyaline to yellow, and plerotic. Sizes range from 19 to 54 µm. All this characters were clearly seen as the laboratory and PDA culture provided artificial environment for growth of *P. cinnamomi.* Naturally, *P. cinnamomi* occurs globally and has a broad host range exceeding1000 plant species (Hardham, 2005), which along with the production of resilient oospores, contributes to its persistence in soils.

5.2 Effects of *P. cinnamomi* and flooding stress on growth and on disease incidence

Phytophthora cinnamomi and flooding had adverse effects on dry weight and other growth parameters such as leaf area, plant height and stem diameter and plant weights. This study has shown a reduction on growth of the four avocado rootstocks when inoculated with *P. cinnamomi* and flooded. The avocado rootstocks showed different growth characters following *P. cinnamomi* inoculation and flooding treatments in agreement to results reported by Pandey *et al*. (2010). Puebla rootstocks had more leaves but with a smaller leaf area. Similar observations were made in Fuerte rootstocks, which may be an adaptation to increase leaf area for the plants to carry out their physiological functions such as photosynthesis as suggested by Reeksting *et al*. (2014). Puebla and Fuerte rootstocks had high leaf number, as well as plant height and stem diameter when compared to the other avocado rootstocks. This suggests faster growth that might have contributed to a larger dry weight. Controversies have previously emerged on plant dry weight under initiated stress. For example, Reeksting *et al.* (2014) found a decrease in the general dry weight with *P. cinnamomi* stress in blueberry genotypes, while Sivaguru and Horst (1993) found it to have no clear effects in apple genotypes. Colmer and Voesenek (2009) found that roots were more sensitive than shoots to *P. cinnamomi* stress in avocado plants. In avocado, Reeksting *et al*. (2014) demonstrated that *P. cinnamomi* affected shoot growth. However, Steward *et al*. (2012) reported that *P. cinnamomi*-tolerant species of avocado maintained relatively high root growth even in soils containing high *P. cinnamomi* concentration and flooding. However in this study growth generally decreased with *P. cinnamomi* and flooding treatment apart from Fuerte which showed a lot of differences in the growth patterns. The reduction in root and shoot dry weights may be due to reduction in leaf expansion which in turn affected the supply of the assimilates to the growing parts of the plant.

Flooding and *P. cinnamomi* inoculation made avocado plants wilt to death faster due to disease incidences as compared to those flooded without inoculation. This means, avocado rootstocks are sensitive to flooding, which leads to decrease in growth in poorly drained soils, leading to leaf premature abscission, root decay, reduced photosynthetic ability and lower enzyme efficiencies. Insufficient oxygen availability in flooded soils also accelerate PRR outbreak of avocado (Fleischmann *et al*., 2002). Flooding and *P. cinnamomi* inoculation had immediate impact on growth of the plants which was greater than when plants were only inoculated with *P. cinnamomi*. In general, significant reductions in dry weights were apparent for rootstock. Reductions in leaves may have caused changes in dry weights of plants that were flooded and inoculated (Reeksting *et al*., 2014). This suggests that changes in carbon allocation are a long term response to flooding in avocado and that reduction first in root dry weights is caused by root rot (Smith *et al*., 2011). Considering all growth parameters determined, significant differences were found among treatments. This suggests a devastating impact on plant health as found by Reeksting *et al*. (2014). These caused disease incidences that were manifested as necrosis, lesion and wilting during combination of flooding and inoculation. Water-logging might have deprived soils with oxygen and allowed motility of zoospore (Pandey *et al*., 2010). Although in some cases un-inoculated plants that were flooded also showed signs of wilting, visible symptoms were severe in Fuerte rootstocks as compared to Booth7, Puebla and Pinkerton rootstocks. Decline in root dry weights under flooded and inoculated treatments may imply root damage and death thereby reducing the sink activity of the roots. Rapid symptoms in all the rootstocks were expected and found to be something normal as avocado plant has been found to be a flood sensitive species (Steward *et al*., 2012).

Leaf wilting, necrosis and decline in general plant health of flooded plants were observed to be high in rootstocks at treatment when compared to controls. Flooding and *P. cinnamomi* inoculation treatment in avocado rootstocks showed wilting due to necrosis lesions. Most of

avocado rootstocks such as Booth 7 and Pinkerton were dead at the end of this study, similar results were reported in variety Duke 7" by Reeksting *et al*. (2014). Non flooded and non inoculated rootstocks appeared healthiest by the end of the study. The inoculated but non flooded avocado rootstocks might have had healthier root systems compared to the flooded and *P. cinnamomi* inoculated rootstocks in agreement with the results by Reeksting *et al*. (2014).

P. cinnamomi inoculation *and* flooding reduced the growth of the avocado plants. This is reflected in the significantly reduced stem diameter, leaf area, plant heights, plant fresh weightsand plant dry weights. Out of the four rootstocks studied, Fuerte and Puebla rootstocks showed more tolerance to *P. cinnamomi* and flooding treatments.

5.3 Influence of *P. cinnamomi* and flooding stress on chlorophyll content andchlorophyll fluorescence

Water-logging and *P. cinnamomi* inoculation induced a decrease in chlorophyll a concentration in avocado rootstocks. This has similarly been reported earlier in other plant species under environmental stresses, such as sorghum (Peixoto *et al*., 2002), beech (Ridolfi and Garrec, 2000) and barely (Abdalla, 2008). It should, however be noted that a decrease in chlorophyll concentration of avocado rootstocks in response to *P. cinnamomi* inoculation and flooding treatments was probably not the primary factor to limit CO_2 assimilation (Jiang *et al*., 2008). A study by Reeksting *et al*. (2014) support this postulate since chlorophyll concentration values were lower in Waterlogging and *P. cinnamomi* inoculation than in control leaves. Reeksting *et al*. (2014) found that a combination of such factors reduced photosynthetic pigment concentration, impaired PSII photochemistry and the distribution of enzymatic machinery accounted for the treatment induced decrease in CO_2 assimilation in avocado rootstocks.

Flooding and *P. cinnamomi* inoculation might have caused a decrease in chlorophyll *a* synthesis in avocado rootstock leaves when compared to the control by inhibiting the activity of -

aminolevulinic acid (-ALA) dehydratase (Pereira *et al*., 2006; Mmayi (2015). Mihailovic *et al*. (2008) found that in stressed sensitive maize inbred line, chlorophyll reduction coincided with 5-ALA synthesis inhibition, chlorophyllase activation and leaf deprivation of Fe and Mg. Therefore decrease in chlorophyll *a* with water-logging and *P. cinnamomi* inoculation for the rootstocks in this study may be attributed to the inhibition of the activity of - aminolevulinic acid (-ALA) dehydratase (Mmayi, 2015).

There was a reduction in chlorophyll *b* concentration under initiation of both water-logging and *Phytopthora cinnamomi* inoculation treatments majorly in Puebla and Pinkerton rootstocks. This may have been due to decreased uptake of Magnessium ions by roots under water-logging and *Phytopthora cinnamomi* inoculation conditions, resulting in a correspondingly decreased PAR utility efficiency which affected the photosynthetic capacity of the rootstocks (Steward *et al*., 2001). The low levels in chlorophyll *a* and *b* decreased total chlorophyll content in avocado rootstocks. Chlorophyll a and b concentration in Fuerte and Booth 7 rootstocks were significantly decreased under flooded and inoculation treatments at the same time. This suggests that there was chlorophyll photo bleaching within PSI and PSII (Reeksting *et al*., 2014) at high rates in these rootstocks, resulting in a smaller fraction of absorbed light energy for electron transport.

Generally plants under water logging and *Phytopthora cinnamomi* inoculation had low chl *a*, chl *b*, and total chlorophyll concentration. *Phytopthora cinnamomi* and water-logging treatments significantly affected the concentration of chl *a*. water logging and *Phytopthora cinnamomi* treatments did not significantly affect the concentration of photosynthetic pigments; chl *b*, chl *a* and chl *a*+*b*.

Phytopthora cinnamomi and flooding treatments affected the photochemical efficiency of Fv/Fm, ΦPSII, NPQ and ETR of the avocado rootstocks investigated differently. In Puebla

leaves, *Phtopthora cinnamomi* inoculation, flooding, and inoculation during flooding caused a significant decrease of the photochemical efficiency of PSII (Chen*et al*., 2005b). Photochemical parameters of PSII have the potential to estimate photosynthetic performance of stressed plants (Maxwell and Johnson, 2000). The Fv/Fm ratio measured in the four rootstocks of avocado after exposure to different treatments showed significant ($p \leq 0.05$) differences. Mean values for maximum quantum yield were high at the control treatment compared to uninoculated-flooded and flooded-inoculated treatments for Fuerte and Booth7 avocado rootstocks. This shows that photosynthetic apparatus of these rootstocks were highly affected compared to Puebla and Pinkerton and that inoculation alone had less effect on the rootstocks similar to findings of Reeksting *et al*. (2014). The Fv/Fm values found in this study did not show a consistent reduction with treatments. According to Kate and Giles (2000), Fv/Fm ratios for normal plants have an optimal value of 0.83. Therefore low Fv/Fm ratios of avocado rootstocks in this study showed that they exhibited normal photosynthesis despite being grown under uninoculated-flooded and inoculated conditions. Fuerte and Booth 7 rootstocks showed abnormal growth with very low values of Fv/Fm that ranged from 2.9 and 0 under flooded and *P. cinnamomi* inoculation conditions. Fv/Fm of plants exposed to a combination of the two stresses (flooding and *P. cinnamomi* inoculation) dropped to level significantly lower than both non-flooded, inoculated plants and control plants. These trends were similar to those reported by Reeksting *et al*. (2014). Fv/Fm ratio is a useful indicator of early responses to flooding or disease infection in plants (Xiao-Bin *et al*., 2007; Maxwell and Johnson, 2000; Marenco and Dias, 2006).

Effective quantum yield (ΦPSII) and ETR had low values at the control treatments compared to uninoculated-flooded and flooded-inoculated treatments of Puebla and Pinkerton rootstocks. The ΦPSII in Fuerte and Booth 7 rootstocks were lower even under the control treatment compared with the other rootstocks. Fuerte and Booth 7 rootstocks may have been intrinsically less efficient at managing their energy for photochemical processes than the other

avocado rootstocks (Giannakoula *et al*., 2008). This rootstock specific behaviour indicates that they might be having lower productivity as compared to the other rootstocks. High values of ΦPSII in Puebla rootstocks showed that the photochemical activity was the main way to dissipate safely the excess energy of excitation. This was an indication that ETR was never saturated showing that other sinks, different from the assimilatory process, were likely to accept electrons (Erwin *et al.* 2014). In this way the excess energy of excitation is dissipated by photochemical activity avoiding the over reduction of PSII reaction centres (Ambrosio *et al*., 2003). Reeksting *et al*. (2014) observed Mehler reaction in mesophyll chloroplasts of C3 species and proposed a role in the production of extra ATP for the pseudocyclic photophosphorylation. Differences in ϕPSII were noticeable at even the onset of visible symptoms between flooded and non-inoculated treatments of both Booth 7 and Puebla rootstocks with flooded and inoculated avocado rootstocks exhibiting lower values, thus highlighting the importance of the combination of the stresses in avocado rootstocks. The reduction in ϕPSII indicates a decrease in the proportions of radiations absorbed by chlorophyll associated with PSII, which is used in photochemistry (Maxwell and Johnson, 2000; Reeksting *et al*., 2014). Decrease in ϕPSII and ETR of avocado rootstocks under this study were not accompanied by an increase in NPQ, possibly suggesting a reduced ability of these plants to dissipate excess energy resulting from a decline in photochemistry.

Thermal energy dissipation measured as NPQ in the four avocado rootstock rootstocks did not have a clear pattern with different treatments although treatment means were significantly different (Appendix 2). In Fuerte, Pinkerton and Puebla cases NPQ was high in flooded-inoculated plants compared to control (Fig. 4.3.4). Less energy was dissipated in treatments of inoculated and un-inoculated flooded, that show the two treatments each to have less effects to growth of avocado plants as compared to inoculated-flooded plants (Lu *et al*., 2003). In this case other metabolic pathways such as the water cycle (Mehler reaction) and

photorespiration in flooded-inoculated avocado rootstocks may have been up regulated to cope with the increased excess of excitation (Reeksting *et al*., 2014) in Fuerte rootstock that had a high NPQ.

It is accepted that PSII is the most vulnerable part of the photosynthetic apparatus to stress-induced damage (Marjorie *et al*., 2010). Inoculated-flooded avocado rootstocks therefore might have used a smaller fraction of the absorbed light in electron transport compared with control leaves which had more excess excitation energy. The main role of NPQ is to indicate dissipation of the excess energy of excitation. The low non-photochemical quenching (Chen and Cheng, 2003) in control plants, indicated that there was less thermal energy dissipation. A higher mean value in flooded and *P. cinnamomi* inoculated avocado rootstocks contributed to excess of thermal energy of dissipation (NPQ). This explains the fact that apart from photochemistry, fluorescence strategy was adopted to dissipate excess energy to some extent. Fuerte avocado rootstocks appeared to have been strongly affected by *P. cinnamomi* and flooding stress since it exhibited high fluorescence and was found to have dissipated more energy.

Generally avocado rootstocks under inoculated and flooded treatments had low values of Fv/Fm, ΦPSII and ETR showing that photosynthetic apparatus were affected by *P. cinnamomi* and flooding treatments. Flooding and *P. cinnamomi* inoculation interfered with chlorophyll fluorescence parameters of the rootstocks. Puebla and Pinkerton rootstocks had high Fv/Fm and ETR values. Booth7 rootstocks behaved differently compared to the rest of avocado rootstocks.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE STUDIES

6.1. Conclusions

i. *Phytophthora cinnamomi* growth on vegetable agar was positive and the pathogen isolated was found to be rosette shaped among other characters and was correctly identified for use in inoculation.

ii. Flooding and *P. cinnamomi* inoculation reduced the growth of the avocado rootstocks. This was reflected in the reduced stem diameter, leaf area, dry weight and relative growth. Puebla rootstock showed more tolerance to *P. cinnamomi* and flooding treatments. Fuerte and Pinkerton rootstocks are considered PRR tolerant rootstock than Booth 7 rootstock, supported by the physiological response of the plants to inoculation by *P. cinnamomi*.

iii. Puebla had high concentration of chlorophyll *a*, *b* and total chlorophyll in the leaves. Generally avocado rootstocks under flooding and *P. cinnamomi* had low chlorophyll a concentration. Avocado rootstocks that were grown under flooded-inoculated treatments had low chlorophyll concentration.

iv. Avocado rootstocks under *P.cinnamomi* and flooding treatments had low values of Fv/Fm, ΦPSII and ETR as an indication that their photosynthetic apparatus were negatively affected. The *P. cinnamomi* did interfere with chlorophyll fluorescence parameters in the avocado rootstocks. Some rootstock differences were evident in Fuerte, Puebla and Booth 7 in Fv/Fm, ΦPSII and ETR values. Pinkerton behaved somehow differently compared to the avocado rootstocks.

6.2 Recommendations

i. Use of a ripe avocado fruit was efficient in isolation of the pathogen but other methods such as the use of leaves can also be tried. PDA growth media and isolation using fruit bating was adequate as the pathogens were isolated and clearly identified to be *P. cinnamomi*.

ii. Plant growth parameters were successfully used in this study to determine the responses of the avocado rootstocks to the flooding and *P. cinnamomi* treatments and they are therefore recommended to be used for determining the effect of *P. cinnamomi* and flooding stress of other avocado rootstocks.

iii. Puebla rootstocks responded better to *P. cinnamomi* inoculation and flooding stress as compared to the other avocado rootstocks. They may be recommended for growing in *P. cinnamomi* infested and waterlogged regions.

6.3 Suggestion for future studies

i. Chlorophyll a/b ratio, carotenoid contents and xanthophyll pigment accumulation can be determined in future to get clear understanding of the correlations between photosynthetic pigments and chlorophyll fluorescence characters.

ii. Additional research is needed to determine the relationships among A, gs, and Ci for more flooded and nonflooded avocado rootstocks inoculated with *P. cinnamomi.*

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APPENDICES

Appendix 1: Analysis of Variance (ANOVA) for plant growth parameters and disease incidence of avocado rootstocks.

Parameter	Source	DF	SS	MS	F	Pr> F
Plant Height	Model	137	475.009496	0.03467223	53.04	<.0001
	Error	54	003.530096	0.00065372		
	Corrected total	191	478.539592			
	Treatment (T)	3	116.549358	0.38849786	594.29	<.0001
	Rootstock (R)	3	120.451942	0.40150647	614.19	<.0001
	T*R	9	102.846492	0.11427388	174.81	<.0001
Stem diameter	Model	11	011.011467	0.01001042	5.51	< 0.0001
	Error	180	032.722500	0.00181792		
	Corrected total	191	043.733967			
	Treatment (T)	3	000.669167	0.00223056	1.23	0.3013
	Rootstock (R)	3	007.174800	0.02391600	13.16	<.0001
	T*R	9	000.008157	0.00000906	0.000	1.0000
Plant fresh weight	Model	11	3231.437218	293.767020	11.10	< 0.0001
	Error	180	4762.702252	26.459457		
	Corrected total	191	7994.139470			
	Treatment (T)	3	1895.781933	631.927311	23.88	<.0001
	Rootstock (R)	3	181.348836	60.449612	2.28	0.0805
	T*R	9	0.943775	0.104864	0.000	1.000

Appendix 1: Analysis of Variance (ANOVA) for plant growth parameters and disease incidence of avocado rootstocks continues.

Plant total dry weight	Model	8	2243.530210	280.441276	4.86	0.0003
	Error	39	2248.149840	57.644868		
	Corrected total	47	4491.680051			
	Treatment (T)	3	424.9929394	141.6643131	10.02	<.0001
	Rootstock (R)	3	191.7069480	63.9023160	4.52	0.0044
	T*R	9	0.7173677	0.0797075	0.000	1.000
Leaf area	Model	8	938.325270	117.290659	4.57	0.0005
	Error	39	1000.905845	25.664252		
	Corrected total	47	1939.231116			
	Treatment (T)	3	73.82955897	24.60985299	2.92	0.0354
	Rootstock (R)	3	89.58627600	29.86209200	3.54	0.0158
	T*R	9	0.02538043	0.00282005	0.000	1.000
Necrosis	Model	8	236.7911326	29.5988916	8.08	< 0.0001
	Error	39	142.8937822	3.6639431		
	Corrected total	47	379.6849148			
	Treatment (T)	3	24.75000000	8.25000000	96.32	<.0001
	Rootstock (R)	3	30.08333333	10.02777778	117.08	<.0001
	T*R	9	31.08333333	3.45370370	40.32	<.0001

Appendix 2. ANOVA for Chlorophyll concentration and chlorophyll fluorescence of avocado rootstocks.

Parameter	Source	DF	SS	MS	F	Pr> F
Chlorophyll *a* content	Model	137	18775.84672	137.04998	3.22	<.0001
	Error	54	2298.42031	42.56334		
	Corrected total	191	21074.26703			
	Treatment (T)	3	6958.927240	2319.642413	54.50	<.0001
	Rootstock (R)	3	1912.771406	637.590469	14.98	<.0001
	T*R	9	2868.255885	318.695098	7.49	<.0001
Chl *b*	Model	15	12301.22744	820.08183	15.14	<.0001
	Error	54	9533.11763	54.16544		
	Corrected total	191	21834.34507			
	Treatment (T)	3	7105.600214	2368.533405	43.73	<.0001
	Rootstock (R)	3	2070.795277	690.265092	12.74	<.0001
	T*R	9	3124.831954	347.203550	6.41	<.0001
Chl *a*+*b*	Model	15	48069.91197	3204.66080	14.96	<.0001
	Error	176	37705.05198	214.23325		
	Corrected total	191	85774.96394			
	Treatment (T)	3	28127.38497	9375.79499	43.76	<.0001
	Rootstock (R)	3	7963.89641	2654.63214	12.39	<.0001
	T*R	9	11978.63059	1330.95895	6.21	<.0001

Appendix 2. ANOVA for Chlorophyll concentration and chlorophyll fluorescence of avocado rootstocks continues.

Parameter	Source	DF	SS	MS	F	Pr> F
FV/FM	Model	137	11.43543953	0.08347036	2.89	< 0.0001
	Error	54	1.55737747	0.02884032		
	Corrected total	191	12.99281700			
	Treatment (T)	3	1.39876196	0.46625399	16.17	<.0001
	Rootstock (R)	3	2.31104987	0.77034996	26.71	<.0001
	T*R	9	4.78912167	0.53212463	18.45	<.0001
ϕPSII	Model	137	11.23668343	0.08201959	2.59	< 0.0001
	Error	54	1.71281305	0.03171876		
	Corrected total	191	12.94949648			
	Treatment (T)	3	1.28920906	0.42973635	13.55	<.0001
	Rootstock (R)	3	2.21704356	0.73901452	23.30	<.0001
	T*R	9	4.08526069	0.45391785	14.31	<.0001
NPQ	Model	137	108.0654880	0.7887992	1.13	0.3106
	Error	54	37.7336486	0.6987713		
	Corrected total	191	145.7991365			
	Treatment (T)	3	1.19746856	0.39915619	0.57	0.6364
	Rootstock (R)	3	3.74254696	1.24751565	1.79	0.1609
	T*R	9	10.19249103	1.13249900	1.62	0.1327
ETR	Model	137	312205.8152	2278.8746	0.53	0.8238
	Error	54	74813.8716	1385.4421		

	Corrected total	191	387019.6868			
	Treatment (T)	3	1.19746856	0.39915619	0.57	0.6364
	Rootstock (R)	3	3.74254696	1.24751565	1.79	0.1609
	T*R	9	10.19249103	1.13249900	1.62	0.1327