YIELD LOSS AND RESISTANCE TO PINEAPPLE HEART ROT DISEASE ON PINEAPPLE CULTIVARS IN CENTRAL UGANDA

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DECLARATION

I, Oculi Jasper, do hereby declare that this is my original work and has never been submitted to any institution of higher learning for an academic award.

Signed.....

Date 07.12.2017

APPROVAL

This is to certify that this work was carried out under our supervision as university supervisors and is now ready for submission for examination.

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DEDICATION

I dedicate this work to my parents Mr. Hannington Okallo, Mrs Joyce Okalo and my beloved children. Without your support and love, this work would not have been possible.

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May the Almighty Lord bless all of you abundantly!

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ACRONYMS

ANOVA:	Analysis of Variance
FAO:	Food and Agricultural Organization
MT:	Metrics tones
PHRD:	Pineapple Heart Rot Disease
RCBD:	Randomized Complete Block Design
CRD:	Completely randomized Design
SAR:	Systematic Acquired Resistance
UBOS:	Uganda Bureau of Statistics
UIA:	Uganda Investment Authority
USDA:	United States Department of Agriculture
AUDPC:	Area under Disease Progress Curve
DI:	Disease index

ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr) is of tremendous importance as a fruit crop in Uganda. However, production in Uganda is currently threatened by outbreaks of pineapple heart rot disease (PHRD). PHRD is the most widespread and devastating disease of pineapple in Uganda and can cause tremendous yield loss. Although, some preliminary information exists on PHRD in Uganda, the yield loss attributed to the disease has not quantified neither is there any information on sources of resistance. The objective of this study was, therefore, to determine yield loss and resistance to pineapple heart rot disease in central Uganda. In order to address these objectives, five cultivars of pineapple were planted in the screen house and in the field in a completely randomized design (CRD) and randomized complete block design (RCBD), respectively. Treatments on yield loss assessment consisted of protected plot, un-protected plot and control plot respectively. Pineapple plants in the protected plots had their suckers dipped for three minutes in a solution of Metalaxyl (Active Ingredients: methoxyacetyl)-N-(2, 6-xylyl)-DL-alaninate 8%) before planting and later sprayed with a solution of Fosetyl Al (Active Ingredients: Aluminum tris 80%) using a backpack sprayer with Hardir flat spray nozzles three weeks after planting. Pineapple plants in the un-protected plots were not treated with any fungicide. The suckers were inoculated with 10^8ml of zoospores using needle-mediated leaf base wound technique. Treatements on resistance consisted of five cultivars of pineapple (Smooth Cayenne, Victoria, Sasilimu, MD-2 hybrid and Red Spanish) Planted in a field with a history of PHRD infestation. Results from the screen house study indicated100% yield loss in all five cultivars after two months. Field experiments indicate that all the five cultivars used were susceptible to PHRD although Smooth cayenne showed moderate resistance to PHRD. Additional studies, therefore, need to be conducted using more pineapple cultivars to establish their suitability as genetic material in breeding for resistance against PHRD and to minimize yield loss.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

1.1.1 Taxonomy and global production of pineapple

Pineapple *(Ananas comosus* (L.) Merr.) is the most economically important plant in the family *Bromeliaceae,* which is divided into three subfamilies: *Pitcarnioideae*, *Tillandsioideae* and *Bromelioideae*. *A. comosus* belongs to the subfamily *Bromelioideae*, order *Bromeliales*, genus *Ananas* and species *comosus* (Bartholomew *et al.*, 2003).The crop is a tropical, herbaceous, perennial monocot, approximately 1-2 meters tall and wide, with leaves arranged spirally. Pineapple bears flowers on a terminal inflorescence, which forms a large, edible fruit characterized by a tuft of leaves at its apex. Pineapple takes 24 months (in the tropics) to 36 months (in the cool subtropical environment) for the propagules to establish into plants and provide fruits (Bartholomew *et al.* 2003). Cultivated pineapple was first described and named *Karatas* and *Ananas* at the end of the 17th century by Charles Plumier on the island of Hispaniola part of Antilles (West Indies). Later, all pineapples were classified in one genus, *Ananas*. Bartholomew *et al* (2003) stated that in 1892 Mez recognized in the *Flora Brasiliensis* only one species *A. sativus* and five botanical varieties. However, pineapple taxonomy underwent further modification several times over the years and it was not until 2003 that the classification developed by Coppens d'Eeckenbrugge and Leal (2003) was internationally adopted. Based on the similarity in floral structure, biology and chromosome number (2n=50), the current classification identifies six botanical varieties of *A. comosus* that intercross successfully with *A. comosus* var. *comosus*to produce fertile offspring (Coppens d'Eeckenbrugge *et al.,* 1997).

According to Bertoni (1919) Pineapple is native to southern Brazil and Paraguay, where the wild relatives occur. The crop is reported to have been domesticated by the Indians and carried through South and Central America to Mexico and the West Indies long before the arrival of Europeans in those areas (Morton, 1987). According to Purseglove (1972) and Bartholomew *et al.* (2003), pineapple was introduced to the east and west African coast by Portuguese traders in 1548 and 1655, respectively. However, by then pineapple was already being grown in South Africa (Morton, 1987). Nevertheless, there is no comprehensive information on when the crop was exactly introduced to Uganda although pineappleis widely grown by small holder farmers across different parts of the country (Ssonko *et al.*, 2003). According to Pickersgill (1976), pineapple became naturalized in many countries including Costa Rica, Guatemala, Honduras, Trinidad and Tobago in the Caribbean and Tropical America as well as other parts of the world. Pineapple is grown commercially in many countries over a wide range of latitudes ranging from approximately 30° N to 33°58'S of equator (Malezieux *et al.,* 2003).

According to Hassan *et al.* (2011; 2015), pineapple is ranked the third most important tropical fruit crop after banana and citrus in the world. In fact, over the last 100 years, pineapple has become one of the leading commercial fruit crops in many tropical countries including Uganda (Menzel, 1994). Globally, over 19 million metric tons of pineapple was produced in 2011 (FAO, 2013). In fact, pineapple contributes over 20% of the world production of tropical fruits (Coveca, 2002). According to FAO (2013), some of the major pineapple producing countries in the world include: Brazil, Thailand, Philippines, Costa Rica and China (Table 1). However, in Africa, Nigeria is ranked as the leading producer of pineapple, with a total production of 1.4 million metric tons (MT), representing about 7% of the global production. Regionally, Kenya is the leading producer of pineapple followed by Democratic Republic of Congo with average

production of 429,065MT and 78,000MT, respectively. Uganda and Rwanda produce 1,600MT and 18,208MT, respectively (FAO, 2013).

Table 1: Some of the world leading pineapple producers

COUNTRIES	PRODUCTION (MT)
Brazil	2,491,974
Thailand	2,278,566
Philippines	2,209,336
Costs Rica	1,678,125
Indonesia	1,550,000
China	1,402,060
Nigeria	1,100,000
Belgium	234,123
Ivory Coast	200,000
United States of America	90,515

Source: FAO 2013

1.1.2 Pineapple production in Uganda

In Uganda, pineapple production has no clearly documented history (Magala *et al.*, 2010). Traditionally, pineapple has been grown for home consumption though in the last two decades, it has assumed commercial importance in some parts of Uganda (Ssemwanga, 2007). According to UBOS (2012), pineapple is the most widely grown commodity in the fruit crop range and value chain. The commercial production corridor for pineapple covers Kayunga, Mukono, Luwero and Masaka districts with Kayunga as the leading producing district in Uganda (Magala *et al.*, 2010). Although, pineapple is a fairly drought resistant crop, a well distributed annual rainfall of at least

1000mm per year is required for high yield (Collins and Baker 1960). According to Bartholomew, (2009), medium altitudes of about 1200m above sea level are the best pineapple producing zones. Below 1200m above sea level, the fruit has little fibre, leading to a mushy fruit and a blunt taste whereas at high altitudes, growth is slow and the fruit is more acidic. (Bartholomew, 2009). Deep sandy loams with a lot of organic matter are ideal for the crop (Collins, 1986).

According to Beauman (2005) between 48–68 pineapples cultivars are recognized globally based on the fruit shapes, flower colour and spininess of the leaf margins. Earlier, Py *et al.* (1987) classified pineapple into five groups namely; Spanish, Queen, Cayenne, Pernambuco and Perolera due to shape, fruit color and leaf arrangement. However, for a very long time, Smooth Cayenne has been the dominant variety in the world until the introduction of MD-2 hybrid variety. According to Bartholomew (2009), the introduction of MD-2 hybrid was a major landmark in the pineapple industry. MD-2 hybrid is a cross between the PRI hybrids 58–1184 and 59–443 (Chan *et al.*, 2003). Accordingly, MD-2 is now grown by many companies and growers around the world. In fact, MD-2 is believed to be the most important pineapple cultivar for the fresh market (Duane, 2009). For instance, it is being exported to many countries including the United States, United Kingdom, Japan, Korea, Hong Kong, China, Singapore and the Middle East (Bartholomew, 2009).

1.1.3 Uses of pineapple

Pineapple is of tremendous importance as a fruit crop in Uganda (UBOS, 2013). Pineapple can be used fresh, juiced, dried, made into candies and incorporated into cooked dishes and desserts (Collins, 1986).The fruit is a good source of potassium, vitamin C and vitamin A (Jonathan,

2013). Fresh pineapple is a good source of carbohydrate, roughages and minerals especially Ca, P, Fe, Na and K. Pineapple is also known to contain some appreciable amounts of vitamins like A, B1, B2, B3, B5, B6, B9 and C (Jonathan, 2013).Unfortunately, the nutritional content of the crop is influenced by several factors including varieties, soil, climatic conditions, maturity stage and handling. According to Saradhuldhat and Paull (2007), processing may result in the nutritional components being altered in the final processed products. However, like many other fruits, pineapple is very low in protein but contains bromelain, a glycoprotein having protease activity commonly used in the food industry for tenderizing meat, therapy for cancer treatment and chill proofing beer (Heinicke and Gortner, 1957; Bhui *et al.*, 2009). Accordingly, bromelain activity remains relatively high during fruit development but declines at the ripening stage along with the total protein content (Gortner and Singleton, 1965; Lodh *et al.*, 1973). Besides, the food uses, pineapple provides fibre that can be used as animal feeds. Additionally, pineapple is used as folk medicine and in some countries as an ornamental plant (Collins, 1960). In some places, the terminal bud or "cabbage" and the inflorescences are eaten raw or cooked. For example, young shoots, called "hijos de pina" are sold in vegetable markets in Guatemala for food (Morton, 1987).

1.1.4 Constraints to Pineapple production in Uganda

Pineapple production in Uganda is beset with myriad of problems including seasonality of production, Pests and diseases, lack of improved pineapple production technologies and declining soil fertility are among the notable constraints (NARO, 2003).

Production statistics in Uganda reveals that the total pineapple production in Uganda in 2014 is 73,400 metric Tons per year and this always leaves a demand gap of about 43,000 metric tons

Accordingly, this implies that despite the market prevalence and the need to increase production, pineapple production remains low (FAO, 2013).

Soil infertility in Uganda has led to low pineapple production. This is especially serious because the already poor soils are constantly under pressure as an increasing number of farmers attempt to make a living based on what the land can offer to grow pineapple. Additionally, the high density of population in the Lake Victoria crescent, an important pineapple production zone in Uganda has led to delicate problems of soil protection against runoff and various types of erosion Land is a major resource in agricultural production. High population growth density in Uganda has caused the shortage of arable land and led to the decrease of farm size and this resulted to the adoption of intensive agricultural practices hence declining of soil fertility. More so, land shortage has increased pressure on the ecosystems and this accelerated the declining of pineapple productivity (NAADS, 2014). Pests, diseases and irregularities in rainfall are al so among the major problems that affect the production of pineapple in Uganda. According to Bua *et al.*, (2013) the common disease in pineapples is Pineapple mealy bug wilt and nematode wilt. Recently, PHRD has been reported to be devastating (Ocwa *et al*, 2016). Moreover, irregularities in rain have impacted negatively towards pineapple production in Uganda. With an economy heavily dependent on rain fed agriculture, climate is of particular importance (Magala *et al*, 2010).

The major constraint in pineapple production in Uganda is the ineffectiveness of the extension system. The extension agents have weak links to the research service; often do not have sufficient means of moving about the countryside, and generally lack knowledge on more specialized topics. They also have a message oriented, top down framework for working and have not developed strong skills as facilitators of the farmers own processes of knowledge

acquisition. Also, there are too few women extension agents. The process of linking District extension agents with specialized sources of knowledge is not well developed (Magala, *et al* 2010).

1.2 Statement of the problem

Yield losses attributed to PHRD in Uganda have not been quantified nor the sources of resistance identified (NAADS 2014; Veerle, 2014). Moreover, no information exists whether variety influences disease severity (Rohrbach and Schenck, 1985). Therefore, assessing the reaction of different varieties to PHRD is crucial because resistance is one of the major components of the integrated disease management package for most plant diseases (Drenth and Sendall, 2004; Gurudatt, 2015). This therefore underscored the need to quantify yield loss attributed to PHRD and identify sources of resistance among the commonly grown pineapple cultivars by farmers in Uganda.

1.3 Justification of the study

Pineapple heart rot disease is a recent outbreak of diseases affecting pineapple production globally (Drenth and Sendall, 2004). For example, in Uganda, PHRD has been reported and confirmed in two major pineapple growing districts of Mukono and Kayunga in the Lake Victoria crescent basin (Bua *et al.,* 2013). In China, PHRD has also been reported to be widespread and devastating, with incidence ranging from 25-30% (Shen *et al.*, 2013). Unless properly managed, the disease can cause significant yield loss (Rohrbach and Schenck, 1985). According to Magala *et al.* (2010), yield loss associated with the disease is important because it influences the choice of management practices. If PHRD is not checked, this is likely to affect communities that derive their livelihood from pineapple through loss of revenue and employment. Additionally, the direct negative, social and environmental impact on the

communities could even be more since a sizable proportion of the vulnerable groups especially women and youths derive livelihoods from pineapple business. Although, PHRD was observed in all fields surveyed in Mukono and Kayunga districts, the incidence and severity varied from field to field (Bua *et al.*, 2013). However, it was also not possible to conclusively estimate the yield loss attributed to the disease although it was evident from the farmers that the disease was widespread and devastating. Additionally, all the five commonly grown cultivars (Smooth cayenne, Red Spanish, Sasilimu, MD-2 and Victoria) appear to be susceptible to PHRD yet no sources of resistance have been reported in the country (Bua *et al.*, 2016). In fact, the alarming rate of spread is a serious threat to food security, livelihoods and loss of pineapple biodiversity. Considering, the fact that farmers do not have any resistant variety as an alternative, the disease is a threat to pineapple production in Uganda if no appropriate control strategy is put in place. The current prescribed preventive and control measures against PHRD have varying effectiveness and sustainability (Green and Scot, 2015). Elsewhere, the major control strategies for PHRD included roguing affected plants, use of chemical fungicides, proper drainage, use of disease free planting materials and addition of manure, among others (Baum & Pinkas, 1988). Unfortunately, these methods do not eliminate the pathogen since it is soil borne and resting spores produced by the causal pathogen (chlamydospores and oospores) may survive in the soil for about 6 and 13 years, respectively (Erwin and Ribeiro, 1996). Accordingly, pineapple heart rot disease is of significant economic importance which requires urgent attention. This study therefore was undertaken to assess the yield loss as well as identify the sources of resistance to PHRD in Uganda.

1.4 Overall objective of the study

The overall objective of this study was to assess yield loss and identify the sources of resistance to pineapple heart rot disease in Uganda.

1.5 Specific Objectives

i. To estimate the yield loss associated with pineapple heart rot disease on five pineapple cultivars grown in central Uganda.

ii. To identify sources of resistance to pineapple heart rot disease on five pineapple cultivars grown in central Uganda.

1.6 Hypotheses

i. Yield of pineapple cultivars in central Uganda is not affected by pineapple heart rot disease.

ii. Resistance to pineapple heart rot disease does not vary among the different pineapple cultivars in central Uganda.

CHAPTER TWO: LITERATURE REVIEW

2.1. Pineapple Heart Rot Disease (PHRD)

2.1.1. Quantification of yield loss in pineapple

Pineapple is susceptible to a number of plant pathogens (Rohrbach and Johnson, 2003). *Phytophthora* diseases are economically important in most pineapple growing regions (Drenth and Guest, 2004). According to Drenth and Guest (2004), the number of *Phytophthora* species attacking pineapple is variable, making it very difficult to assess the economic impact of disease on pineapple. Additionally, the relationship between rots and yield loss are not proportional. According to Rohrbach andSchenck (1985), losses due to *Phytophthora* vary a lot with seasonal and climatic conditions. This is especially true for heart rot which can lead to serious losses under wet conditions while being virtually absent in years with below-average levels of rainfall. *Phytophthora* infect the roots, cherelles, pods, roots, stems, and leaves of many plants including pineapple (Thurston, 1984). Drenth and Sendall (2004) noted that when environmental conditions are optimum for disease development, the entire disease cycle may occur in 1-2 weeks. According to James and Scot (2015), many plants including pineapple attacked by *Phytophthora* root rot do not show aboveground symptoms until summer. However, as the hot, dry weather sets in, the plant does not have enough functional roots left to keep up with transpiration. In fact, the plants frequently wilt and collapse within a week. Because of the wilting, many people water plants even more than usual, flooding their roots, encouraging the pathogen, and potentially spreading the disease even more leading to significant yield loss (Rohrbach and Shenck, 1985).

2.1.2 Symptoms of Pineapple Heart Rot Disease

Pineapple heart rot disease manifests as water-soaked tissue on the center most leaves surrounding the apical meristem, formation of brown streaks on lamina and in mesophyll tissues (Drenth and Sendall, 2004). Light brown exudates emerge from the blisters as leaves begin to rot (Shen *et al.,* 2013). A few days after initial infection of the apical meristem and lateral buds, the pineapple heart and stem can be easily detached from the below ground portion of the plant (Drenth and Sendall 2004).

2.1.3 Economic importance

Pineapple heart rot disease is widespread and devastating in many pineapple growing countries. For instance, in China, the incidence of PHRD ranged from 25-30% (Shen *et al.*, 2013) this represents significant loss in yield as infected plants collapse and die. Losses from PHRD can be severe in poorly drained fields. Plants on even relatively well drained soils can be affected during prolonged wet weather. Losses from root rot can be serious in high rainfall areas where prolonged rains extend into the winter months. The disease can eliminate the ratoon crop. Rough leaf varieties and some low acid hybrids like Red Spanish were reported to be more susceptible than Smooth Cayenne in a study conducted in Kerala agricultural university pineapple research station, India (Joy and Sindhu. 2012). It was also reported that the traditional variety of pineapple was already suffering losses from PHRD though this loss was not quantified. A report by FAO (2007) in Hawaii indicated that pineapple production in 2007 dropped from 212,000 tons in 2005 to 172,500 tons in 2007 due to PHRD. According to Drenth and Guest (2004), *Phytophthora* heart rot disease caused by *Phytophthora cinammomi, P. nicotinae and P. palmivora* is the most destructive disease of pineapple worldwide. Annually, the disease results in estimated losses in pineapple production in Asia, Africa and Brazil totaling to about 450,000 tons, worth 423 million

United States dollars in revenue. However, the impact of *Phytophthora* disease varies from country to country and in different crops.

Rots caused by *P. palmivora and P. nicotianae* have also been reported in Malaysia on palm tree and pineapple, respectively (Waller and Holderness, 1997). The diseases are responsible for yield reduction due to premature nut fall, root and heart rots. Bud rot, heart rot and nut fall caused by *P.palmivora and P. nicotianae* were first reported in Indonesia in 1985(Bennett *et al.*, 1986). The outbreaks of these diseases resulted in severe damage to plantations with MAWA palm germplasm and Red Spanish pineapple, respectively (Renard, 1992). In fact, almost all areas planted with MAWA coconut and pineapple in Indonesia have suffered serious damage from bud rot and heart rot with losses in excess of 80% (Darwis, 1992). In some areas, stand losses of 43% can occur due to bud and heart rot on palm tree and pineapple, respectively. Premature nut fall, heart and fruit rots, which is the most common disease of palm and pineapple affects nuts of 3–7 months old and pineapple plants at all stages of growth (Lolong *et al.*, 1998) and can cause losses of 50–75% in both crops (Brahamana *et al.*, 1992).

Phytophthora capsici, also referred to as "sudden wilt", causes foot rot of black pepper. According to Holliday and Mowat (1963), an epidemic of the disease in Sarawak in the mid-1950s caused crop losses of almost 100%. Similarly, crop losses of 40–50% due to root rot have been recorded in other areas (Erwin and Ribeiro, 1996). In other countries, yield losses are higher (10–15%); in Vietnam they are higher still (15–20%) due to inexperience in managing root rot, high ground water tables in some areas and the use of susceptible varieties (Erwin and Ribeiro, 1996). In the USA, the yield losses by *Phytophthora* disease on pineapple were estimated to range from 3–6% a year. On the other hand, in the wet tropical areas of Southeast

Asia, the yield loss is estimated to be 6–12% a year due to extensive monsoonal wet periods which favor disease development (Drenth and Guest, 2004).

2.1.4 Disease management

Prophylactic sprays of 1% Bordeaux mixture, metalaxyl (Ridomil) and Fosetyl Al (Aliette) have been used in controlling the root rot phase in pineapple (Whiley *et al.*, 1986). According to Rohrbach and Shenck (1985), both fungicides have been reported to control heart rot on Pineapple in other countries. When applied either as a pre-plant dip on vegetative seed material or as a post plant spray, Fosetyl Al reduced mortality caused by heart rot and has been recommended for the control of these diseases (Pegg, 1977). While, Bordeaux mixture preparation and spraying during rainy season is a cumbersome process, it has also been reported to cause copper toxicity to grazing animals (Rohrbach and Shenck, 1985). Control of the disease by spraying fungicides from the early stages of plant development until flower initiation is largely used (Matos, 2009), but frequently without taking into account the possible risks to human/ animal health and the environment. Furthermore, pathogen populations with resistance to the fungicide have been reported (Ventura *et al.*, 1981). In recent years, numerous reports on promising biological control agents have been published for different pathosystems (Chen *et al.*, 2015; Luambano *et al.*, 2015). According to Druzhinina *et al.*, (2011), Fungi in the genus *Trichoderma* are the most studied among the fungal agents to control plant diseases, including PHRD, worldwide. Nevertheless, there are few studies on the yield loss and resistance to the disease in general and none in Uganda.

Phytosanitary measures have been recommended as a cost effective way of controlling many diseases, including PHRD (Smith, 1988). In his findings, hygienic precautions can be applied to exclude *P. cinnamomi* from a place of production. The author empathized that unsterilized soil or

growing medium, or farm machinery, should not be brought in. Similarly, introduced plants should be kept apart until their phytosanitary status has been checked. All propagation should be done from healthy plants or seed. Cultural measures should be taken to reduce the risk of spread in case of introduction. For example, when the fielddoes become infected, incidence of *P. cinnamomi* can be reduced if not necessarily eliminated by leaving the land under non-susceptible crops for at least 4 years and by applying various control measures such as field hygiene, use of healthy planting materials among others (Baum and Pinkas, 1988). Additionally, cultural control measures including alleviation of high soil moisture levels, improving aeration by increasing drainage and managing mineral nutrition and elements of the soil micro flora were recommended (Stirling *et al.*, 1992). It was demonstrated that the above cultural practices suppressed *P. cinnamomi* in some soils and may be a potential cultural control agent for the control of the disease (Sarpong, 2016). Soil solarization was also reported to control *P. cinnamomi* on young avocado plants and pineapple (Kotzé and Darvas, 1983). However, Coffey (1987) elucidated in his findings that an integrated approach is generally taken to control *P. cinnamomi* on avocado and pineapple.

2.2 Resistance to pineapple heart rot disease

Resistance has been advocated as the most economical and cost- effective alternative, for farmers, in controlling plant diseases (Van der planck, 1963; Parveliet *et al*, 1996; Agrios, 1997; Drenth and Guest, 2004; Agrios, 2005). In fact, several recent studies have shown that a diverse genetic basis of resistance is beneficial for the farmer because it allows a more stable management of pest and disease pressure than monoculture allows (Trutmann *et al.*, 1993; Thurston *et al.*, 1999;Thinlay *et al.*, 2000; Finckh, 2003; Di Falco and Chavas, 2007; and Jarvis *et al.*, 2007). However, the effectiveness of resistance levels depends not only on the availability

of resistant genes but also on the nature and speed of the life cycles of pathogens as well as their means of spread (Chakraborty *et al.*, 1991; Finckh *et al.*, 2000). *Phytopthora*, which causes heart rot disease in pineapple, vary in the degree of host specificity. For instance, *P. fragariae* var. *rubi* infects a single host species (Kennedy and Duncan , 1995), while *P. cinammomi* is able to attack over 1000 different host plant species (Erwin and Ribeiro, 1996); other *Phytopthora* species occupy a continuum between these two extremes (Zentmyer, 1983). Elsewhere, Rodriquez *et al.* (2002) indicated that *Bromelia penguin,* one of the cultivars of pineapple was resistant to *Phytophthora nicotianae* var. *parasitica* compared to other varieties like Serrana, Red Spanish Enana, Spanish Nozera´n Red, Spanish Colorada Caney, Red Spanish Pinaren˜a, and Red Spanish Colorada Ramo´n which were all susceptible to *Phytophthora nicotianae* var. *parasitica* and died. According to Green and Scot (2015), no superior pineapple varieties with acceptable resistance to PHRD are available. 'MD-2', a hybrid of predominantly 'Smooth Cayenne' parentage, exhibits the greatest resistance when environmental conditions favor plant growth, but not when conditions favor the pathogen.

According to Itefnet (2016), the local clones of Smooth Cayenne locally called 'Sarawak'are usually resistant to fruit collapse and bacterial heart rot diseases but susceptible to black heart disorder due to low temperature. Also, Spanish cultivars are reported to be susceptible to fruit collapse, bacterial heart rot diseases and PHRD.

Hybrid 36 is a hybrid selected from a cross between 'Gandul' (Red Spanish) and the Smooth Cayenne by the Peninsula Estate, Malaysia. It is a very robust cultivar which is quite tolerant to black heart disorder but susceptible to marbling diseases and PHRD (Itfnet, 2016).

˜Josapine' is a new cultivar in the Spanish group with very bright prospects as a table-fruit. It is a selection from hybridization between 'Johor' (Spanish) and 'Sarawak' (Smooth Cayenne) and released by the Malaysian Agriculture Research and Development Institute (MARDI) in 1996. 'Josapine' pineapple is susceptible to PHRD and resistant to black heart disorder or internal browning caused by low temperatures.

"Maspine" is originally known as Line 73-50, a complex hybrid from five types of pineapple which seldom has stem, aerial and ground shoots. "Maspine" belongs to "Manzanah" group that inherits the vigor of "Queen", high sugar content from "Cayenne", golden yellow aril and fruit shape of "Singapore Spanish", resistant to disease from "Pernambuco" and non-spiny leaves from "Perolera". It is reportedly resistant to Bacterial Heart Rot (BHR) and PHRD. "Maspine" has high potential for planting on mineral soils which is already infected by BHR and PHRD.

Pernambuco' ('Eleuthera') and Mordilonais a pineapple cultivar whose cultivation is restricted to certain South American countries like Brazil, Ecuador, Colombia, Venezuela and Peru. The cultivar is resistant to *fusariosis* disease and commonly used as a parent in breeding programs.

The cultivars'Queen' (also called 'Common Rough' in Australia, Phuket', ˜Rough McGregor', ˜Ripley Queen', ˜Alexandra' and ˜Victoria' in other parts of the world) are widely distributed and quite extensively cultivated for fresh fruits because of its high sugar content and unsuitable canning qualities, Queen cultivars are robust and show higher tolerance to stress and pests than 'Smooth Cayenne'. However they are susceptible to PHRD and black heart disorder.Singapore Red' (Also called 'Red Jamaica', 'Singapore Spanish', 'Singapore Queen', 'Singapore Common) is disease and pest-resistant (Itfnet, 2016).

Additionally, Gevens *et al.* (2006) reported resistance of cucumber to *P. capsici*. In their findings, it was observed that younger, smaller plants were comparatively more susceptible than older, larger plants. The potential of the genetic resistance as a control measure for the pineapple *fusariose* has been detected both under field conditions and under controlled conditions using artificial inoculation techniques (Cabral *et al.*, 1985; Matos *et al.*, 1991). Differences in pineapple cultivar resistance to fusariose in the field in the presence of natural inoculums were observed by Giacomeli and Teofilo (1984). Inoculation studies provided evidence for genotypes resistant to the disease (Cabral *et al.*, 1985, Cabral and Matos, 1989; Cabral *et al.*, 1991; Matos *et al.*, (1991). The identification of sources of resistance to *Fusarium subglutinans* has been commended as a very important step in obtaining pineapple varieties resistant to major pathogens of pineapple (Cabral and Matos 1995). Growing resistant varieties, besides being the most efficient control measure for several plant diseases, is also an environmentally safe approach (Matos, 2006). The basis of this study was therefore to screen pineapple germplasm for possible resistance to PHRD in central Uganda.

2.3 Assessment of yield loss by pineapple heart rot disease

There exists little doubt that the need for disease assessment and yield loss in the world food crops' including pineapple is of paramount importance (Cook, 2006). The importance of accurate disease assessment was identified by Chester (1950) and Large (1966). Kranz (1988) stated that without quantitative assessment of disease, then no studies in epidemiology, no assessment of crop losses and no plant disease surveys and their applications would be possible. However, the relationship between phytopathometric data and yield loss has always suffered from a number of confounding factors and as a result, many authors, including James (1983), have criticized the lack of reliable estimates of crop yield loss assessment due to plant diseases.

Madden and Nutter (1995) discussed the importance of accurate disease assessment. Additionally, Gaunt (1995) reviewed technologies in disease measurement and yield loss assessment, Gaunt (1995) elucidated that assessment of the amount of disease on a plant or crop is essential in any quantitative epidemiological study.Similarly, Jones and Clifford (1978) and James (1983) identified a number of important reasons for quantitative assessment of diseases and crop loss measurements, the most important of which is decision making on costs of management.

Campbell and Maidden (1990) pointed out that a successful system for disease assessment gives accurate, precise and reproducible results. To that effect, disease can be measured using direct methods by assessing disease in plant or plant material. Additionally, indirect methods, through monitoring spore populations using spore traps can be used. Traditional methods of disease assessment, such as the use of pictorial keys derived from standard area diagrams to evaluate disease severity on a 0–100% scale, have now been joined by several new approaches made possible by rapid advances in computer technology (Tomerlin, 1988; Cooke, 2006). In addition, modern assays using immunological and molecular techniques for the identification, detection and quantification of plant pathogenic organisms are used (Madden and Hughes., 1999). Other new approaches to phytopathometry have evolved, in which remote sensing, image analysis and the detection of crop stress caused by disease using changes in chlorophyll fluorescence and foliage temperature are involved (Nilsson,1995). Obviously, direct methods are likely to be more strongly correlated with yield losses in crops and therefore to be preferred. Indirect quantitative disease assessment has been widely used in fruit crops such as pineapple and is largely concerned with measurements of incidence or severity (Cook, 2006). Disease incidence is a

binary variable, that is, a plant unit is either visibly diseased or not (Madden and Hughes., 1999). Disease incidence would be suitable for assessing systematic infection which may result in total plant loss such as PHRD. Additionally, disease incidence may also be useful in early stages of an epidemic caused by a pathogen when both incidence and severity are related and increase simultaneously (James, 1983). Another method of disease assessment is the use of pictorial disease assessment keys on leaves, fruits or whole plants (Campbell and Madden, 1990).These measurement methods, however, suffer from fundamental errors. Cook (2006) noted fatigue, time of the day, and numbers of experimental units among other factors that may seriously reduce the accuracy of disease incidence as an assessment method in virtually all crops including PHRD. Diagrams on the other hand do not display the variegated patterns of disease, thus an observer is compelled to visualize the total area of infection combined and expressed as a percentage area of plant affected by the disease. Variation of affected parts for example roots of pineapple, leaves and fruits affected by PHRD vary in sizes hence affecting assessment using pictures. Nutter and Schultz (1995) concluded that the accuracy and precision of disease assessment can be improved simply by selecting the most appropriate methods and by training observers to assess disease using computerized disease assessment programmes such as AREAGRAM, DISTRAIN and DISEASE.PRO. These programmes, however, generate only standard area diagrams with fixed disease patterns (Shane *et al.*, 1985).

Quantitative approaches, involving comparing yield from protected plots with yield in unprotected plots, remain the simplest method of assessing yield loss in any patho-systems including pineapple (Bandana *et al.*, 2014). Cook (2006) pointed out that for quantitative approach to be used as a tool in assessing disease impact, it is important that the experiment be

properly designed so that result can be analyzed statistically and if possible, they should be repeated over a number of seasons and in different areas. Quantitative methods, thus, formed the basis of this study.

2.4. Literature summary

Phytophthora heart rot disease in Uganda is caused by fungus, *Phytophthora nicotianae* belonging to oomycetes (Ocwa *et al*., 2017). Plants of all ages are attacked, but three to four month old crown are most susceptible (James and Scot, 2015). Chlamydospores of *P. nicotianae* are the primary inoculum and they can survive in the soil or in infected plant debris for several years (Joy and Sindhu. 2012).Yield losses from PHRD elsewhere have been reported to reach up to 100% where control approaches are poorely followed (Drenth and Guest, 2004). However, no similar studies have been done in Uganda to assess yield loss caused by PHRD. Additionally, no pineapple cultivars have been identified with acceptable level of resistance to PHRD (James and Scot, 2015).

CHAPTER THREE: QUANTIFICATION OF YIELD LOSS DUE TO PINEAPPLE HEART ROT DISEASE IN UGANDA

3.1 Introduction

Pinapple heart rot disease is a destructive disease causing losses in several parts of the world (Shen *et al.,* 2013; Rodríguez *et al.*, 2015; Ocwa *et al.*, 2016). The economic losses by the pathogen are devastating (Rodriguez *et al.*, 2015). In Uganda, pineapple heart rot disease caused by *Phytophthora* is reported to be wide spread in the central districts of Masaka, Mukono, Luwero and Kayunga (Ocwa *et al.*, 2016). The information on yield loss and resistance to PHRD in Uganda is scanty (NARO, 2012). Knowledge of yield loss and sources of resistance is fundamental in designing tangible disease management approach (Agrios, 2005). The objective of this study was therefore to assess yield loss caused by PHRD in five pineapple cultivars in central Uganda.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted in the screen house at the Department of Agriculture, Kyambogo University between March and September 2016. Kyambogo University is located approximately 8 kilometers, by road, east of Kampala's central business district. The elevation and coordinates of KyambogoUniversity are: (1189 meters above sea level, 00°20′54″N, 32°37′49″E) (https://en.wikipedia.org/wiki/2015/Kyambogo_University/Kyambogo -location- Elevation-coordinates/) .

3.2.2 Planting materials

Five pineapple cultivars namely Smooth Cayenne, Victoria, Sasilimu, MD-2 hybrid and Red Spanish obtained from farmers' fields were used for the study. The five cultivars were selected

because they are the only common cultivars in the study location. Cultivar smooth Cayenne is however the most preferred in the local and domestic market therefore grown by most farmers compared to the other four cultivars (NARO, 2012). The disease free status of the planting materials (suckers) was confirmed by planting the suckers in the pots filled with 10 kilograms of sterilized clay loam soils in the screen house for three months from November, 2015 (Shen, 2012). The plants were watered as and when necessary. Suckers that showed symptoms of PHRD were uprooted and discarded. Suckers that did not show signs of PHRD were deemed disease free and subsequently use for the trial.

3.2.3 Inoculum preparation

One hundred twenty (120) symptomatic pineapple samples were collected in the month of November, 2015 from the districts of Mukono, Kayunga, Luwero and Masaka. The collected samples were packed in paper bags and taken to the laboratory for isolation of possible causal pathogens.Isolation of pineapple heart rot disease causal organisms was done using cornmeal agar amended with 10mg pimaricin, 250mg ampicillin, 10mg rifampicin, 10mg benomyl, 25mg Pentachloronitrobenzene (PCNB) and 50mg hymexazol (PARBPH) as described by Drenth and Sendall (2001). The symptomatic pineapple leaves were washed under running water to eliminate soil. 5mm pieces were cut off the disease lesions between healthy and diseased tissue. The cut tissue pieces were disinfested by immersion in a solution of 70 % ethanol for 3 minutes, rinsed three times with sterile distilled water and dried with sterile paper towels. The dried leaf fragments were placed on cornmeal agar (CMA) amended as described above (Drenth and Sendall 2001; Mounde *et al.,* 2012; Rodriguez *et al.,* 2015). The petri-plates were incubated at 24°C in the dark for 2-3 days (Drenth and Sendall 2001; Mbaka *et al.,* 2010; Mounde *et al.*, 2012). Pure cultures of *Phytopthora* species were obtained by sub-culturing hyphal tips on to

freshly prepared corn meal agar (ACMA) for 2-3 days. *Phytophthora* isolates used for the study were induced to sporulate following the protocol described by Jeffers (2006). Zoospore release was induced by incubating agar plugs with sporangia in non-sterile soil extract solution (NS-SES) at 4°C for 30minutes to shock the sporangia. Isolates were later placed at room temperature for 10-20 minutes to burst the sporangia so as to release zoospores (Saadoun and Allagui, 2008).The zoospores were observed on a light microscope to confirm their presence, and subsequently, the number of zoospores was quantified using haemocytometer (Matos, 2006; Cabral, 2012). (Plate 1). The pathogenicity of *Phytophthora* isolates recovered from the infected plant tissues was confirmed by inoculating them on two month old healthy MD-2 pineapple plants (Shen *et al.*, 2013).

Plate 1 A: Pure culture of PHRD ready for quantification with haemocytometer **B:Assessing the presence of zoospores using light microscope**

3.1.4 Experimental layout

The experiment was arranged in a completely randomized design (Plate 3) with three replications.

Plate 2: Heat sterilization of soil

Plate 3: Pineapple cultivars planted in a screenhouse, Kyambogo in a completely randomised design at Kyambogo University screen house, 2016

Treatments consisted of protected plot, un-protected plot and control plot respectively. Pineapple plants in the protected plots had their suckers dipped for three minutes in a solution of Metalaxyl (Ridomill) following the manufacturer's specification before planting and later sprayed with a solution of Fosetyl **Al** (Aluminum tris 80%) using a backpack sprayer with Hardir flat spray nozzles three weeks after planting. Pineapple plants in the un-protected plots were not treated with any fungicide. Each pot was planted with a single pineapple plant, with 50 pots constituting

an experimental unit in a plot. Actively growing pineapple plants in both protected and un-protected plots, two month old were then inoculated with 10^8 ml of the sporangia suspension of PHRD causal pathogen using the needle-mediated leaf base wound technique (Rodriquez *et al.* 2002) Plate.4. Control plots were inoculated with 10ml of sterile distilled water. The inoculated plants were incubated for 2 days under saturated moist condition in the screen house (Jee *et al.*, 2000). Disease symptoms appearance was monitored on weekly basis for two months.

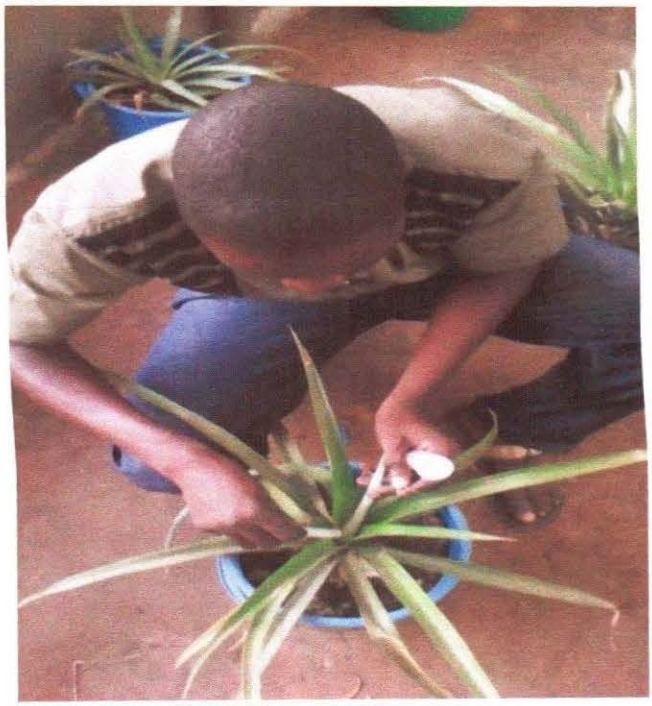


Plate 4: Needle-mediated leaf base wound inoculation

3.1.5 Data collection

Data was collected commencing on symptoms appearance. Data was collected from all the plots and thus used to compute yield loss per cultivar. The formula of Teshome and Tegegn (2017) was adopted with modification to estimate yield loss per cultivar in each plot. Disease incidence was assessed as the number of diseased plants expressed as a percent of the total number of plants assessed per replication. Disease severity was assessed on individual plants by examining

symptoms on the inoculated plants using a modified rating scale of 0-5: where 0=no visible symptoms, 1=early rot with 1-10% leaves affected, 2=mild rot

with 21-40% of leaves affected, 3=average rot with 41-60% of leaves affected, 4=severe rot with 61-80% of leaves affected and 5=pineapple base completely rotten with 100%, leaves affected causing the death of the plant (Souza *et al.*, 2011, Rohrbach and Schenck, 1985).

3.1.6 Yield loss estimation

The relative losses in pineapple yield were determined as a percentage of that of the protected plot. Losses were calculated separately for each of the five pineapple cultivars using the formula below:

$$RL\ (\%) = \frac{(Y_1 - Y_2)}{Y_1} \times 100$$

Where,

RL% = percentage of relative loss (reduction of the yield)

Y1 = mean yield on the protected plots (plots with fungicide treatment)

Y2 = mean yield on unprotected plots (plots without fungicide)

3.1.7 Data Analysis

Analysis of variance (ANOVA) of the Genstat computer program (15th edition) was used to test for the significance of treatments on pineapple heart rot disease and associated yield loss across the different cultivars used in the experiment. Differences in means were separated using Least Significant Difference (LSD) test at 5% probability level.

3.2 Results

Data collected on PHRD incidence and severity, trial two is presented in Fig 1, 2 and Appendix 1, 2, 3& 4. Incidence of PHRD in week 1 ranged from 0.0 to 2.89 for Smooth Cayene and MD-2

hybrid, respectively. However, there was significant difference in incidence btween Smooth Cayene, Sasilimu and Red Spanish, respectively. However, by week 8, there was no significant difference ($P>0.05$) in disease incidence among all the cultivars. Overall, the lowest and highest disease incidence in week 1 and week 8 was recorded in Smooth Cayenne, Sasilimu and MD-2 hybrid and Smooth Cayenne and Red Spanish, respectively (Appendix 3) Relatedly, PHRD severity in week 1 was 3.22 in MD-2 hybrid, 3.33 in red Spanish, 0.0 in Sasilimu, 0.0 in smooth cayenne and 3.11 in Victoria. However, in week 8, severity of PHRD was 33.33 in MD-2 hybrid, 33.67 in red Spanish, 31.0 in Sasilimu, 33.28 in smooth cayenne and 30.67 in Victoria respectively (appendix 4). Additionally, variety effect did not influence PHRD incidence in week 1, week 6 and week 8. However, in week 2, week 4, and week 7, variety significantly ($p<0.001$) influenced incidence of PHRD. Similarly, fungicide treatment significantly ($p<0.001$) reduced the incidence of PHRD from week 1 up to week 8 of the trial (appendix 1).

Variety did not influence PHRD severity in week 3, week 5, week 6 and week 8, but was significant ($p<0.05$) in week 1, week 2, week 4 and week 7 respectively. Fungicide treatment significantly ($p<0.001$) reduced PHRD severity in all the pineapple cultivars used in this trial from week 1 up to week 8 (Appendix 2)

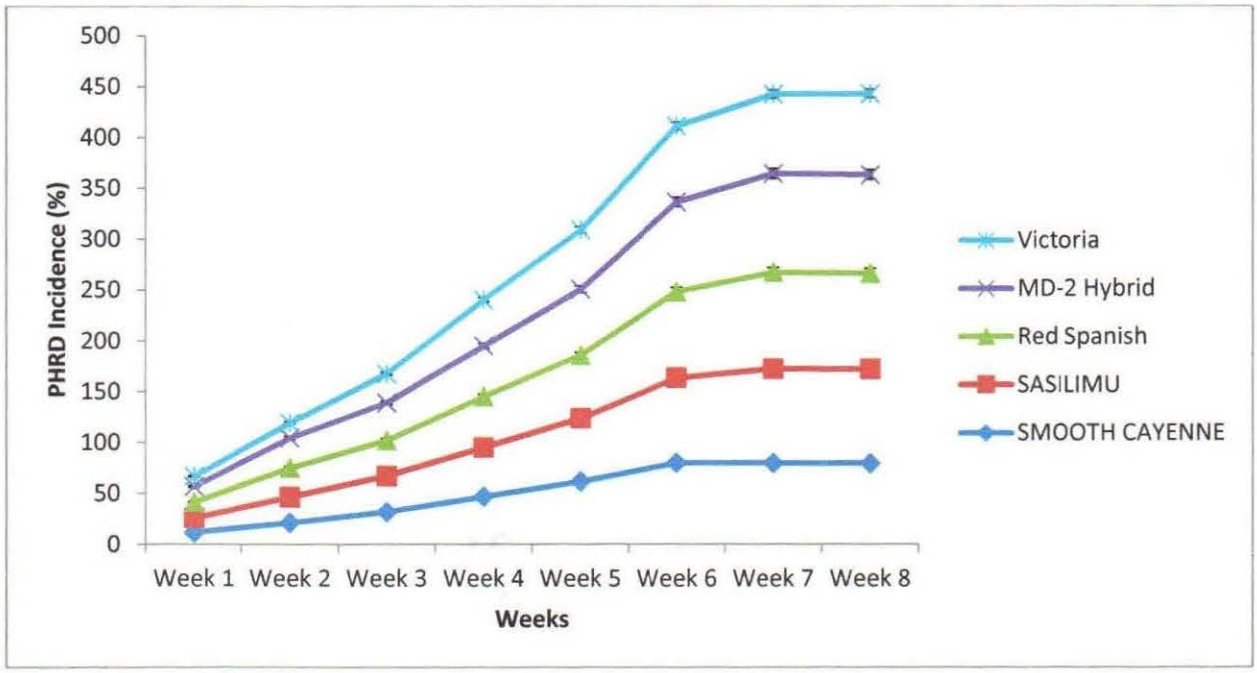


Figure 1: Incidences of PHRD, trial 2 in an evaluation done in screen house, department of Agriculture, Kyambogo University, 2017

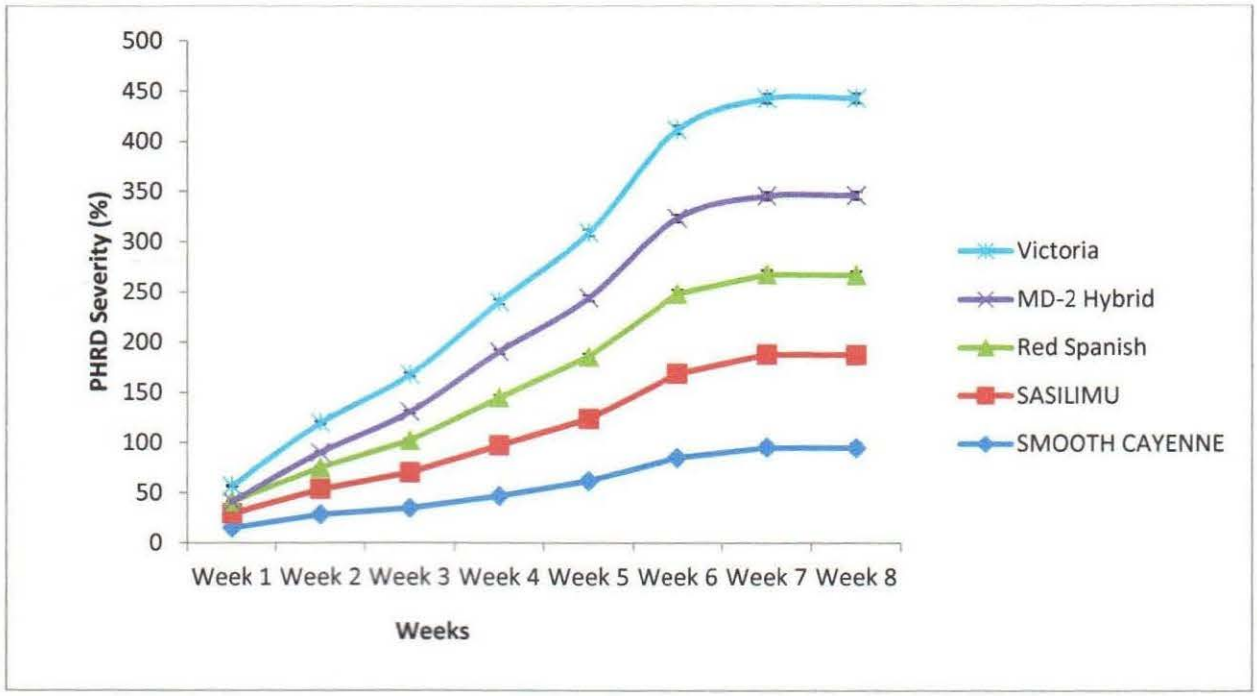


Figure 2: Severity of PHRD, trial 2 in an evaluation done in screen house, department of Agriculture, Kyambogo University, 2017

Data collected on PHRD incidence and severity, season 1 is presented in Figure 3 and 4 and Appendix 5and 6. Incidence of PHRD in week one was at 66.7% in MD-2 Hybrid, Red Spanish and Victoria. Sasilimu and Smooth Cayenne recorded low incidence of 55.6% and 32.3% respectively. Variability was observed in severity among the cultivars in week one with MD-2 hybrid, Red Spanish, Victoria, Sasilimu and Smooth cayenne recording severity score of 5.94%, 6.56%, 4.445, 3.285 and 5.0% respectively. There was no variation of incidence in week seven from week one in all the three cultivars except smooth cayenne and Sasilimu. Severity increased progressively in all the five cultivars from week one to week eight. However Red Spanish had the highest severity score of 6.56% in week one while smooth cayenne recorded the lowest severity score of 3.28% in week one. MD-2 hybrid, Victoria and Sasilimu recorded severity scores of 5.94%, 5.0% and 4.44% respectively in week one. In week eight, the highest severity score was recorded in Victoria at 37.56% and the lowest score was in smooth cayenne at 26.94%. Red Spanish recorded 36.33% while MD-2 hybrid and Sasilimu recorded 35.94% at week eight. Incidence of PHRD was significant ($p<0.001$) in all the cultivars from week 1 to week 4 and week 8 respectively. However, there was no significant relationship recorded on incidence of PHRD in all the cultivars from week 5 week 6 and week 7. Fungicide treatment significantly ($p<0.001$) reduced incidence of PHRD in all the five cultivars used in the trials from week 1 up to week 8 (Appendix 6). Similarly, severity of PHRD did not differ significantly ($p>0.05$) in all the five cultivars in week 1 and week 8. In addition, a significant ($p<0.001$) relationship was recorded in severity of PHRD in all the five cultivars from week 2, 4,5,6,7 and week 8 respectively. On the other hand, Fungicide treatment significantly reduced PHRD severity in all the five cultivars from week 1 up to week 8 (Appendix 7).

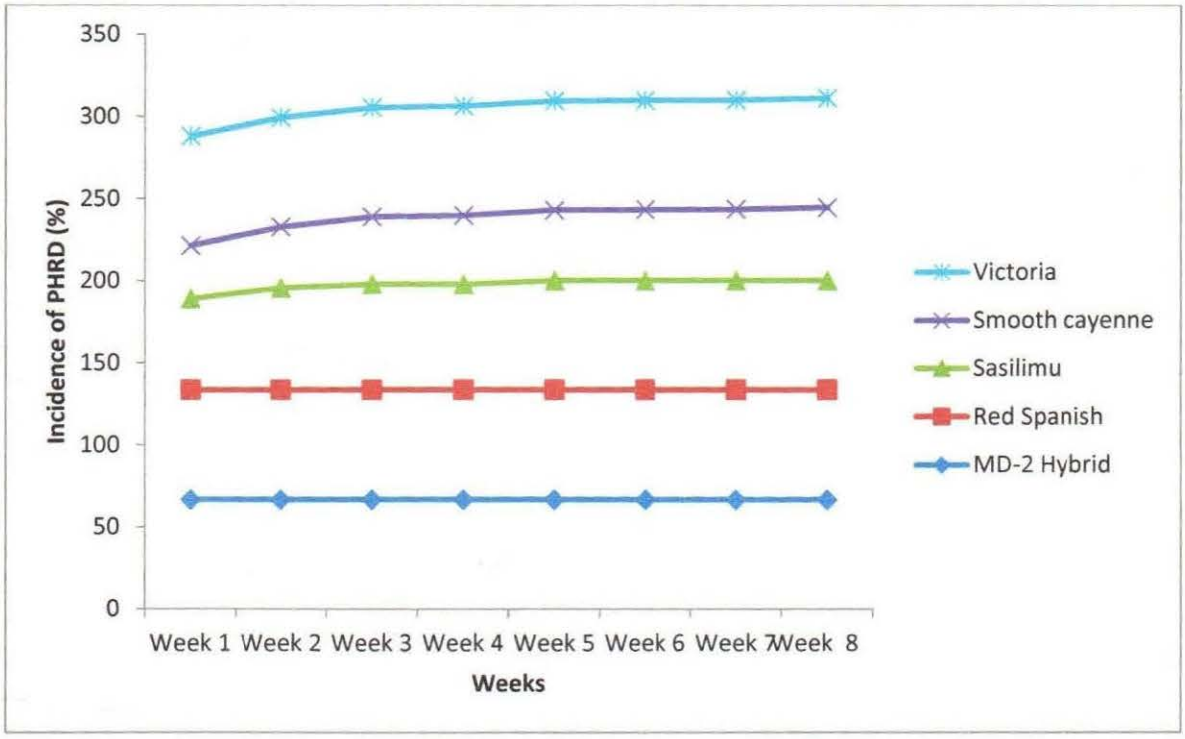


Figure 3: Disease incidence of PHRD, trial 1 in the screen house Kyambogo University, 2016

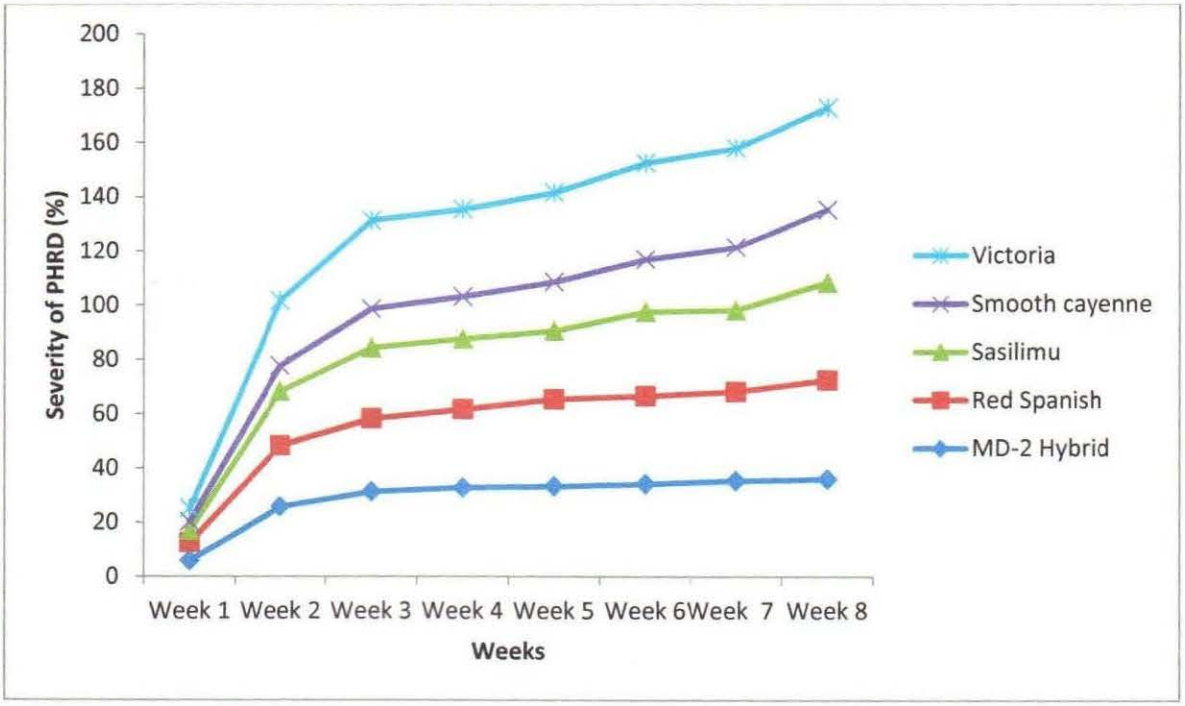


Figure 4: Disease severity of PHRD, trial 1 in the screen house, Kyambogo University, 2016

Table 2 present statistical analysis of yield loss in season 2 among the five cultivars used. MD-2hybrid, Red Spanish and Victoria had the highest yield losses of 99.72%, 99.67%, 99.44%, 97.28%, 99.83% respectively. No yield loss was recorded in protected plot and control plot respectively.

Table 2: Yield loss per variety, trial 2 at an evaluation done at Kyambogo University screen house, 2017

VARIETY	YIELD LOSS IN PROTECTED PLOT	CONTROL PLOT	YIELD LOSS IN UNPROTECTED PLOT
MD-2HYBRID	0	0	99.72
RED SPANISH	0	0	99.67
SASILIMU	0	0	99.44
SMOOTH CAYENNE	0	0	97.28
VICTORIA	0	0	99.83
MEANS			**99.19**
LSD			**2.110**

Table 3 present's statistical analysis of yield loss in season 1 among the five cultivars used. MD-2hybrid, Red Spanish and Victoria had the highest yield losses of 100% respectively, although Sasilimu and smooth cayenne recorded 90% and 80% mean yield loss in unprotected plot respectively. No yield loss was recorded in protected plot and control plot.

Table 3: Yield loss per variety, trial 1 at an evaluation done at Kyambogo University screen house, 2016

VARIETY	**YIELD LOSS IN PROTECTED PLOT**	**CONTROL PLOT**	**YIELD LOSS IN UNPROTECTED PLOT**
MD-2HYBRID	0	0	100
RED SPANISH	0	0	100
SASILIMU	0	0	90
SMOOTH CAYENNE	0	0	80
VICTORIA	0	0	100
MEANS			**31.3**
LSD			**33.14**

3.3 Discussion

The results of this study have shown significant variation in incidence and severity of PHRD under different treatments during the trials. Yield loss varied across different treatments. There was no loss in yield in protected plot and control plots across all the cultivars evaluated compared to 100% yield loss in unprotected plot recorded by MD-2hybrid,Victoria and red Spanish, although Sasilimu and smooth Cayenne recorded yield of 10% and 20% respectively in the unprotected plot for trial 1. In trial 2 however, MD-2hybrid, Victoria, red Spanish, Sasilimu and smooth Cayenne recorded yield loses of 99.72, 99.67, 99.44, 97.28, and 99.83 respectively. The yield recorded by Sasilimu and smooth Cayenne in unprotected plot in trial 1 and the subsequent yield recorded by all the varieties in trial 2 could be as a result of disease escape, or resistance. The low incidence of PHRD recorded on pineapple treated with Fosetyl Al and Metalaxyl in both trials is in agreement with the findings of Farih *et al*, (1981) who found that high concentration of the two fungicides of up to 1 g/L resulted in effective control of PHRD. According to Rohrbach and Shenck (1985), both fungicides have been reported to control heart rot on Pineapple in other countries. When applied either as a pre-plant dip on vegetative seed material or as a post plant spray, Fosetyl Al reduced mortality caused by heart rot and has been recommended for the control of these diseases (Pegg, 1977). Also Matos *et al.* (2009), further demonstrated that PHRD can be effectively control by spraying with fungicides from the early stages of plant development until flower initiation. Accordingly, Joy and Sindhu (2012) recommended the use of systemic fungicides to reduce heart rot disease problems. In their findings, this program should start with the treatment of planting material before planting. After planting, drenching or spraying with registered fungicides at recommended rates and intervals is necessary to control yield losses. This is consistent with the result of this finding where plots

treated with Ridomil as pre-plant dip and later sprayed with Fosetyl Al 3 weeks after planting did not register any yield loss.

Additionally, in China, the incidence of PHRD ranged from 25-30% (Shen *et al.*, 2013). This represented significant loss in yield as infected plants collapse and die. Losses from PHRD can be severe in poorly drained fields. Plants on even relatively well drained soils can be affected during prolonged wet weather. Losses from root rot can be serious in high rainfall areas where prolonged rains extend into the winter months. This is because the zoospores of *Phytophthora* heart rot disease pathogen move about freely in free water around the pineapple roots causing severe damage (Drenth and Guest, 2004; Joy and Sindhu 2012). The report by FAO (2007) in Hawaii indicated that pineapple production dropped from 212,000 tons in 2005 to 172,500 tons in 2007 due to PHRD, this is consistent with our results. The findings by Drenth and Guest (2004), are consistent with the result of this study, in their report, *Phytophthora* heart rot disease caused by *Phytophthora cinammomi, P. nicotinae and P. palmivora* is the most destructive disease of pineapple worldwide. The report indicated that annually, the disease results in estimated losses in pineapple production in Asia, Africa and Brazil totaling to about 450,000 tons worth 423 million United States dollars in revenue. Additionally, a study conducted by Darwis (1992) is validated by the result of this study. The results of our findings compares very well with that of Darwis (1992) who observed that PHRD is a serious constraint to pineapple production in Indonesia where loses of between 80-100 % was documented.

CHAPTER FOUR: EVALUATION OF PINEAPPLE CULTIVARS FOR RESISTANCE TO PINEAPPLE HEART ROT DISEASE

4.1 Introduction

Pineapple heart rot disease is reported to be caused by a number of P*hytophthora* species (Rohrbach and Johnson, 2003). However, the species of P*hytophthora* causing serious destruction in Uganda has been identified as *Phytophthora nicotianae* (Ocwa et al., 2016) Resistance has been advocated as the most economical and cost- effective alternative, for farmers, in controlling plant diseases (Drenth and Guest, 2004; Agrios, 2005). In fact, several recent studies have shown that a diverse genetic basis of resistance is beneficial for the farmer because it allows a more stable management of pest and disease pressure than monoculture allows (Jarvis *et al.,* 2007). However, the effectiveness of resistance levels depends not only on the availability of resistant genes but also on the nature and speed of the life cycles of pathogens as well as their means of spread (Chakraborty *et al.*, 1991; Finckh *et al.*, 2000). The basis of this study was to evaluate pineapple cultivars in central Uganda for resistance to PHRD.

4.2 Materials and methods

4.2.1 Experimental site and lay out

This experiment was conducted in Nazigo sub-county, Kayunga district from May, 2016 to September 2016. Kayunga district is located approximately 74 kilometers (46 mi) northeast of Kampala, on an all-weather road. The district lies on the coordinates 01 00N, 32 52E. (Google map, 2015). The field where the experiment was set had a history of severe PHRD infection (plate 5)

Plate 5: Infected field Site in Kayunga

4.2.2 Sample collection and preparation

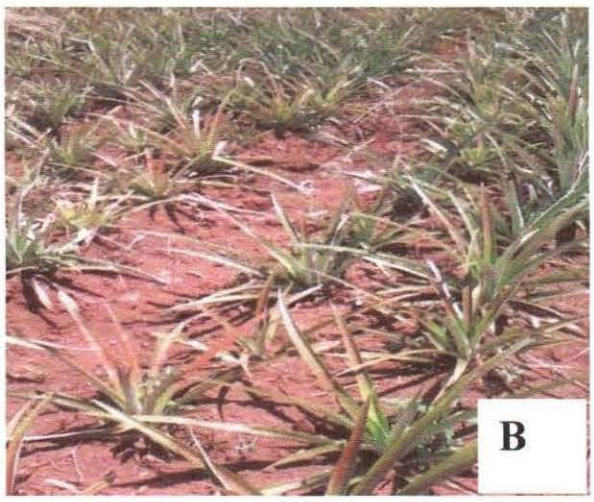
Disease free pineapple suckers of the five cultivars including Smooth Cayenne, Victoria, MD-2 hybrid, Red Spanish and Sasilimu collected from the districts of Masaka, Luwero, Mukono and Kayunga were used for the study. Disease free suckers were purposively picked from the four districts. Fields with history of PHRD were avoided. The disease free status of the planting materials(suckers) was confirmed by planting the suckers in the pots filled with 10 kilograms of sterilized clay loam soilsin the screen house for three months from November, 2015 (Shen, 2012) plate 6. The plants were watered as and when necessary. Suckers that showed symptoms of PHRD were uprooted and discarded. Suckers that did not show signs of PHRD were deemed disease free and subsequently use for the trial in Kayunga district.

Plate 6 : Pineapple cultivars on trial for latent infection before planting in the field
A: One week after planting **B: Two month after planting**

4.2.3 Experimental site preparation

The experimental design was a randomized complete block design (RCBD) replicated four times. Each replicate block comprised of 100 pineapple planted in double rows. Overall 00 pineapple plants of the five varieties were used for this trial. The experimental plots was deep ploughed and harrowed to obtain the right tilth (Plate 7). Each plot measuring 4 m×2m represented a replicate with treatments. The plots within the block were separated by an alley of 1m while the block by 2m (Banful *et al.,* 2011). Five pineapple cultivars (smooth cayenne, Victoria, MD-2 hybrid, red Spanish and Sasilimu) of average sizes were planted in double rows at the spacing of 45x30cm on 26th May, 2016 (plate 7 B). Each replicate bed was mulched with *Paspalum* spp.grass after planting to help retain moisture in the soil, reduce nutrient leaching (especially nitrates) and to control weed growth on the beds (Nakasone and Paull, 1998). Natural infection was allowed to take place. All normal agronomic practices for pineapple cultivation were followed (Nakasone and Paull, 1998).

A



B

FIELD LAYOUT

C

REP 1	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC
	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC
	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS
REP 2	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS
	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC
	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC
	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
REP 3	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC
	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS
	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC
REP 4	SM	SM	SM	SM	SM	SM	SM	SM	SM	SM
	SC	SC	SC	SC	SC	SC	SC	SC	SC	SC
	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS
	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC	VIC
	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD

SM Sasilimu SC Smooth Cayenne RS Red Spanish VIC Victoria MD MD-2 Hybrid

Plate 7: A. Land preparation B. pineapple cultivars C. Field lay out .

4.2.4 Data collection and analysis

Data collection on PHRD incidence and severity commenced two weeks after planting and continued on a fortnightly interval for a period of three months, or until final disease severity in the plots reached 100%. Disease index (DI) was assessed after 90 days. In each sample unit, the

suckers were pulled out from the soil and the internal symptoms were assessed by measuring the size of the lesion in relation to the infected heart leaves (Souza et al., 2011).

Disease incidence was assessed as the number of diseased plants expressed as a percent of the total number of plants assessed per replication. Disease severity was assessed on individual plant using a modified rating scale of 0-5: where 0=no visible symptoms, 1=early rot with 1-10% leaves affected, 2=mild rot with 21-40% of leaves affected, 3=average rot with 41-60% of leaves affected, 4=severe rot with 61-80% of leaves affected and 5=pineapple base completely rotten with 100%, leaves affected causing the death of the plant (Souza *et al.*, 2011; Rohrbach and Schenck, 1985).

Resistance, susceptibility and tolerance was determined using a modified scale of 0-4 where: 0= 1-19% (resistant); 1.0 = 20-40%= (moderately resistant); and 2=41-60% (moderately susceptible); 3= 61-80% (susceptible); 4= 81-100 (highly susceptible) (Souza *et al.*, 2011). Cultivars in which infection took place but the colonization of the host tissue proceeded slowly, reaching a scale of 1 or less 2 months after natural infection and which showed significantly less disease severity (40% below) were considered as resistant to PHRD (Matos *et al.*1991).

Analysis of variance (ANOVA) of the Genstat computer program was used to test for the significance of disease incidence and severity across the different varieties used in the experiment. Differences in means were determined at 5% significance level.

4.3 Results

4.3.1 Screening for resistance to PHRD in Kayunga

There was varying levels of susceptibility to PHRD among the five pineapple cultivars in season 2 trial (Figure. 5 & 6). The highest and lowest incidence of PHRD one week after planting was

recorded on MD-2 hybrid and Smooth Cayenne with 3.992% and 0.491%, respectively. However, by week twelve after planting, there was varying levels of susceptibility with MD-2 hybrid recording the highest incidence of 88.1 and smooth Cayenne recorded low incidence of 52.5%. Red Spanish, Sasilimu and Victoria registered incidence of 78.7%, 75.0% and 81.8% respectively in week 12 (Figure 5). Correspondingly, the highest and lowest PHRD severity one weeks after planting was recorded on MD-2 hybrid and Smooth cayenne with 7.58% and 1.08% respectively. However, twelve weeks after planting, MD-2 hybrid, red Spanish, Smooth Cayenne Sasilimu and Victoria registered severities of 98.9, 90.3, 61.6, 88.6, and 94.2 respectively. (Figure.6). Disease index score in week twelve was 99.75%, 97.67, 77.0%, 96.67 and 99.83% in MD-2 hybrid, red Spanish, Smooth cayenne Sasilimu and Victoria respectively. Overall, no significant ($p>0.05$) relationship in PHRD incidence was recorded in week two and week 4 of the experiment. However, there was significant relationship ($p<0.001$) in incidence of PHRD from week 6 up to week 12 of the experiment. Additionally, PHRD severity varied significantly ($p<0.001$) from week 2 up to week 12 (Appendix 5).

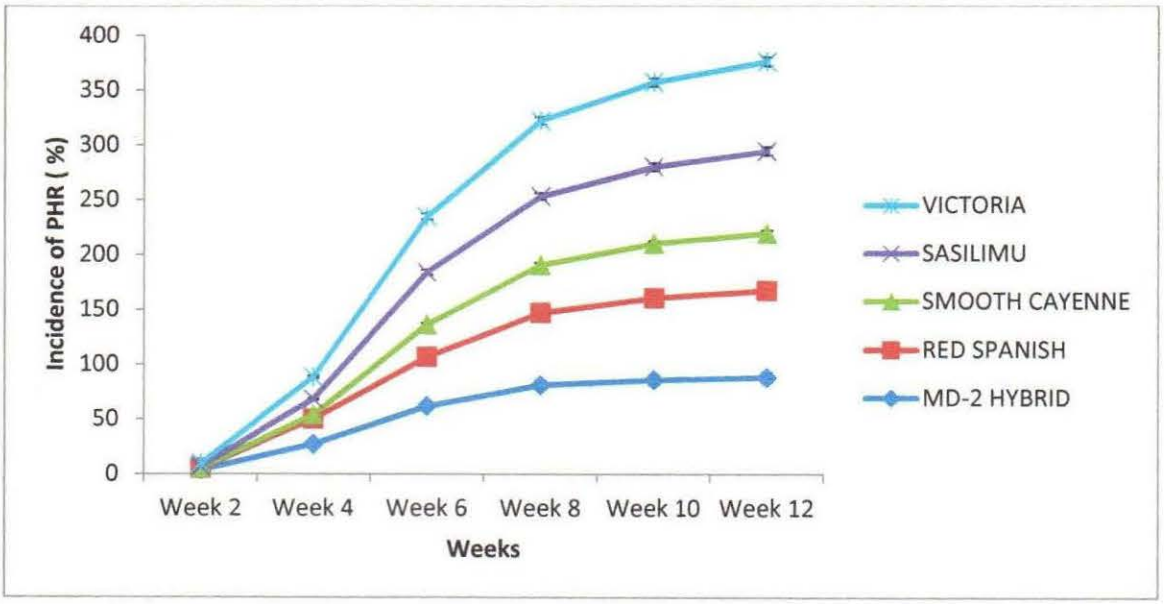


Figure 5: Mean incidence of PHRD, Season 2 in an evaluation at Kayunga, 2017

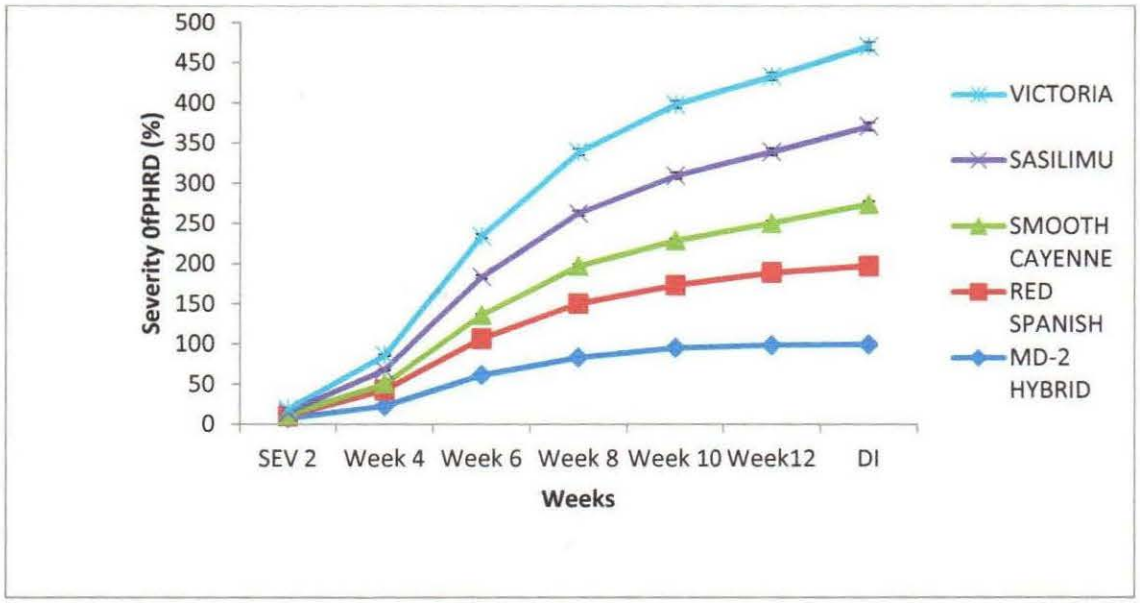


Figure 6: Mean severity of PHRD, season 2 in an evaluation at Kayunga, 2017

There was varying levels of susceptibility to PHRD in season 1 among the five pineapple cultivars three month after planting (Figure 7, 8, 9; plate 8 and 9). The highest and lowest incidence of PHRD one week after planting was recorded on Victoria and Smooth Cayenne with 18.8% and 0% respectively. However, twelfth week after planting, all the varieties recorded 100% incidence except smooth Cayenne and Sasilimu with incidences of 95.62% and 99.75% respectively (Table 6). Correspondingly, the highest and lowest PHRD severity one week after planting was recorded on Victoria and smooth Cayenne with 9.1% and 0% respectively. However, twelve weeks after planting, all the cultivars recorded high severity (Table 7 and Figure 5). Smooth Cayenne and Sasilimu recorded severity of 77.7% and 99.5% respectively in week twelve. MD-2 hybrid, red Spanish and Victoria recorded 100% disease severity in week twelve. Disease index score in week twelve was 72.2% and 95% in smooth cayenne and Sasilimu respectively. MD-2 hybrid, red Spanish and Victoria recorded 100% disease index at week twelve. Overall, no significant relationship in PHRD incidence and severity was recorded

from week two of the experiment ($p>0.05$). However, there was significant relationship ($p<0.001$) in incidence and severity among the varieties from week four up to week twelve (Appendix 10).

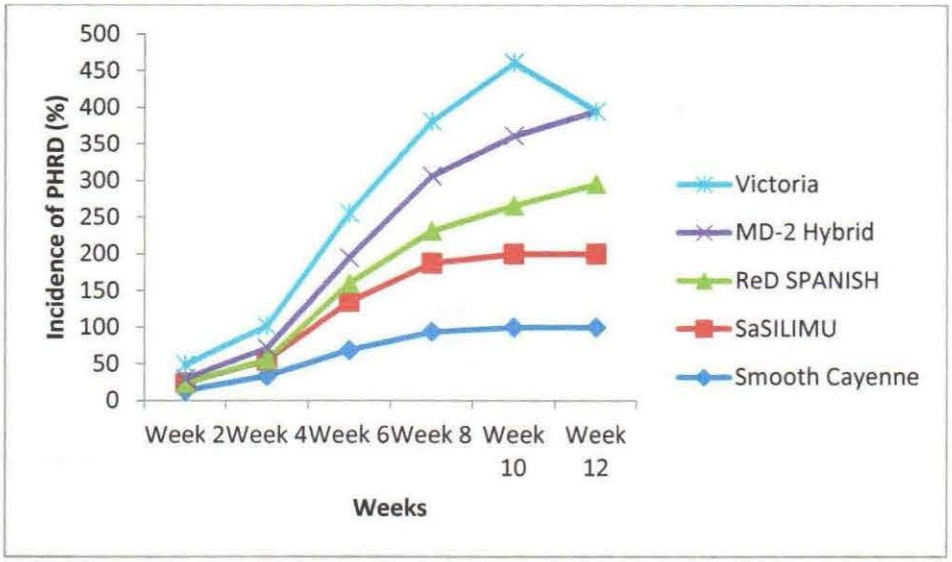


Figure 7: Mean incidence 0f PHRD, Season 1 in an evaluation at Kayunga, 2016

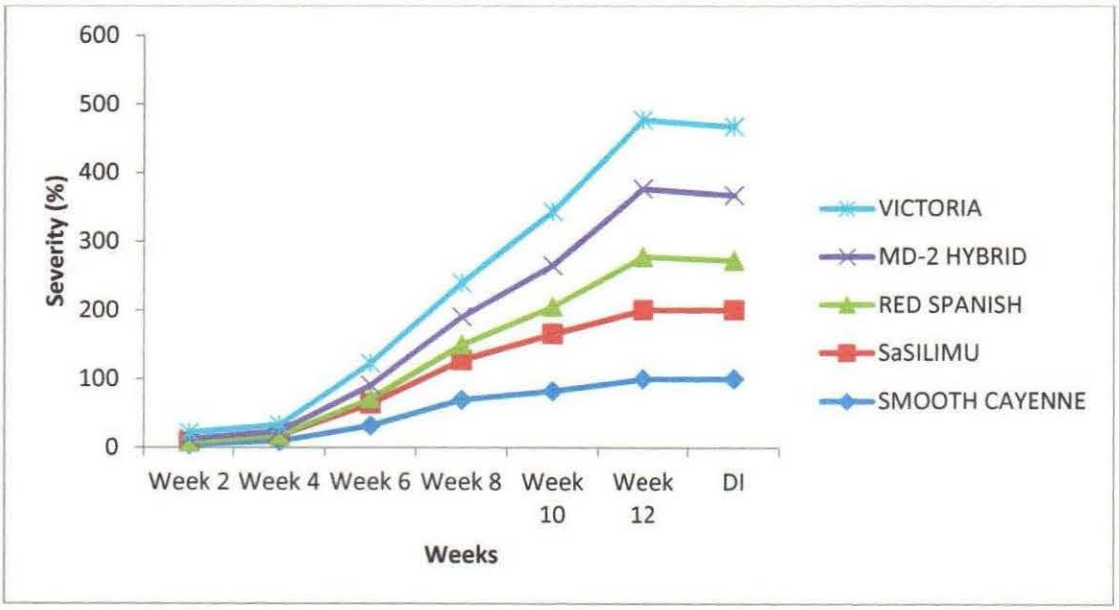


Figure 8: Mean Severity and Disease Index of PHRD, Season 1 in an evaluation at Kayunga, 2016.

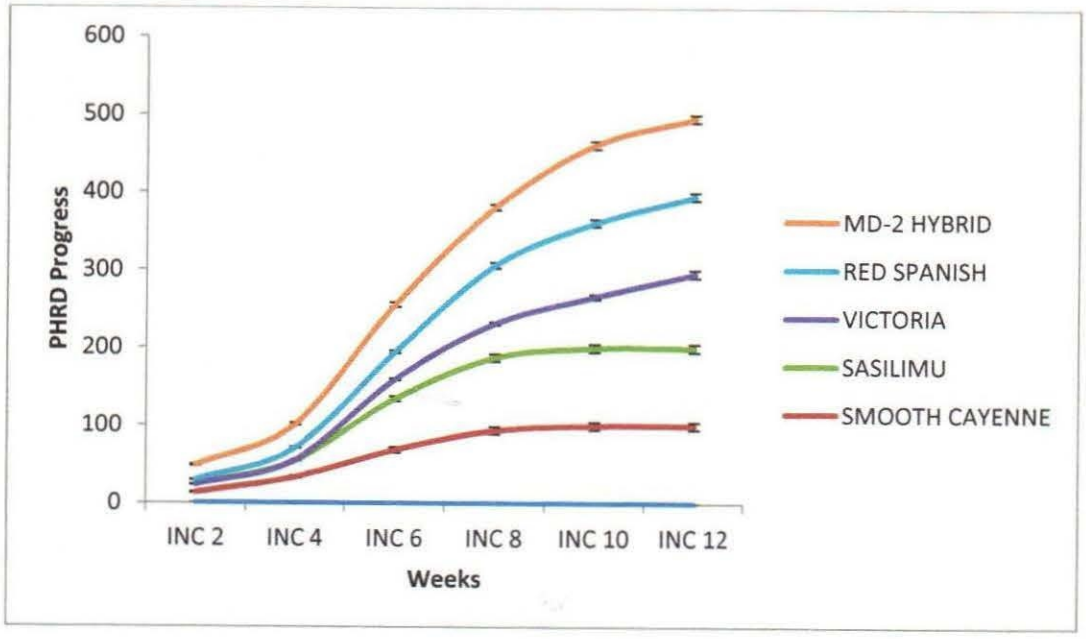


Figure 9: Disease progress on 5 pineapple cultivars, season 1 in an evaluation at Kayunga, 2016

Plate 8: Infected pineapple plants, eight weeks after planting in an evaluation at Kayunga, 2016

Plate 9: Ratoon of smooth Cayenne three months after infection of parent suckers in Kayunga, 2016

4.4 Discussion

The results indicated that all the cultivars were susceptible to PHRD although smooth Cayenne showed some resistance. This could be as a result of the genetic composition of the cultivars with limited resistant genes against the pathogen for PHRD. This is supported with the findings from Green and Scot (2015) who concluded that superior pineapple varieties with acceptable resistance to PHRD are not available. Additionally, the quantity of the inoculum in the experimental site could have resulted in high levels of susceptibility. This is particularly possible since the site had history of severe PHRD. This compares very well with the findings of Rodriquez *et al.* (2002) who reported that cultivars such as Serrana, Red Spanish Enana, Spanish Nozera´n Red, Spanish Colorada Caney, Red Spanish Pinaren˜and Red Spanish Colorada Ramo´n were all susceptible to *Phytophthora nicotianae var.parasitica* in the field with history of the same disease. Similarly, variability in the levels of severity across different varieties used in this experiment does not confirm resistance to the same disease. This therefore is in line with

the report of Drenth and Guest, (2004) who indicated that breeding to increase levels of non-specific resistance does not completely stop infection and colonization but slows down the rate of spread of an epidemic. Additionally, Drenth and Guest (2004) pointed out that resistance may not necessarily give high levels of control to PHRD. Without a more complete understanding of disease cycles of the pathogen and various expressions of disease resistance in different tissue of the host plant, they further pointed out that it is difficult to make significant steps forward by focusing on isolated aspects of resistance in the host plant.

The high level of susceptibility to PHRD registered by the five pineapple cultivars evaluated in this study could also possibly be as a result of the conditions created by the agronomic and production systems used over the years in the field where the trial was conducted. This is in agreement with findings from Rohrbach *et al.* (2003) who noted that commercial pineapple production systems have contributed to the severity of several pest and disease including PHRD. In the same study, Rohrbach *et al.* (2003) pointed out that pineapple plants are subject to a minimum of pests and diseases if proper care and pest management practices are employed. In addition, diseases like heart rot, root rot, fruit rot and butt rot were reported as major problems during handling, storage or planting of fresh materials (Rohrbach & Schmitt 1994). Relatedly, Rohrbach & Schmitt (1994) reported in their findings that cultivar Smooth Cayenne is sensitive to diseases like mealybug wilt, fusariosis, fruitlet core rot, butt rot and internal browning, but resistant to heart rot disease which is consistent with the result of this study.

In spite of the high level of susceptibility recorded in this field trial, cultivar smooth cayenne showed some resistance to PHRD. This resistance could be due to the fact that Smooth Cayenne exhibits strong apical dominance. This is because stem weight of smooth cayenne gradually and progressively increases from the time of planting, plants accumulate starch reserves in the stem

and the amount of starch varies with the environment, thus becoming more resistant to disease attack compared to other cultivars (Bartholomew *et al.* 2003). Similarly, climatic conditions determine active growth of the plant and hence resistance to both biotic and abiotic stress (Bartholomew *et al.* 2003), thus it can be postulated that the climatic condition in the experimental site was more favorable to cultivar Smooth cayenne and less favorable to the rest of the cultivars evaluated in this trial. Additionally, Py *et al.* (1987) and Lim (1985) noted that cultivar smooth cayenne is resistant to fruit collapse caused by *Erwinia Chrysanthemi* Burkbolder and *Phytophthora* sp.which concretizes the results of this study.

It is the practice in commercial cultivation of pineapple to plant one cultivar in a monoculture system (Sanewski & Scott 2000; Bartholomew *et al.* 2003). In Kayunga, only one cultivar of pineapple (smooth cayenne) locally known as *Nnalongo* is almost exclusively cultivated. The related cultivars are not present in the pineapple agricultural ecosystems in Kayunga. Therefore intraspecific gene transfer can potentially only occur within this variety/cultivar and its clones. This partly explains why smooth Cayenne, a dominant cultivar in the study location had moderate resistance to PHRD.

The fungus that causes PHRD is a slow growing pathogen with a slow rate of spread (Drenth and Guest 2004). In light of the fore mentioned, smooth cayenne could have escaped the virulent stage of this pathogen resulting in the resistance recorded in this study. This is in agreement with the study conducted by Chakraborty *et al.*, (1991); Finckh *et al.*, (2000) who postulated that effectiveness of resistance levels depends not only on the availability of resistant genes but also on the nature and speed of the life cycles of the pathogens as well as their means of spread. Differences in pineapple cultivar resistance to PHRD in this trial compares very well with findings from Giacomeli and Teofilo (1984) who pointed out differences in pineapple resistance

to *fusariosis* in the field in the presence of natural inoculum. Additionally, inoculation studies provided evidence for genotypes resistant to *fusariosis* disease in the pineapple field (Cabral *et al.*, 1985, Cabral and Matos, 1989; Cabral *et al.*, 1991; Matos *et al.,* (1991). Similarly, the effects of zoospore concentration in the experimental field could have influenced resistance among the pineapple genotype used in this study. For instance, inoculum concentration was estimated at 8^8 zoospores ml^{-1} which is too low to cause 100% mortality in a variety like smooth cayenne with some resistance. This confirms findings from Rodriquez *et al* (2002) who's findings indicated that the highest rates of plant death were observed with the use of 10^8 zoospores ml^{-1} resulting to 100% plant death after infection. In vitro results from the same study confirmed the same results under field conditions.

CHAPTER FIVE: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General Discussion

The results from this study showed that all the pineapple genotypes used in the experiment were susceptible. This could have been because of the high disease pressure and conducive environmental conditions in the field. This is in agreement with findings from Hu (2007), who postulated that *Phytopthora* pathogens occupy a wide ecological habitat and are very destructive to pineapple. Severity of PHRD varied across genotypes. Smooth Cayenne recorded low incidence and severity in the first weeks of the experiment. This could have been because of disease escape, non -specific resistance or both. This confirms earlier findings by Lim (1971) who indicated that in subtropical climates, where PHRD is a problem, the resistant 'Smooth Cayenne' cultivar might be used rather than the much more susceptible 'Spanish' types. This implies that smooth Cayenne could be used as a resistant genotype in the cultural control of PHRD in Uganda. According to Drenth and Guest (2004), integrated disease management strategies should address numerous components of the disease cycle through selecting disease-resistant planting material, and preventing or disrupting the dissemination of primary inoculum from the soil into the suckers and the movement of secondary inoculum from one part of the field to another. Drenth and Guest (2004) however noted that there is still considerable scope for research into several aspects of the resistance of pineapple and the genetics of the pathogen, especially in understanding mechanism of pathogenesis, host specificity of *Phytophthora* pathogens and in the development of sustainable and cost-effective integrated disease-management practices.

Crop losses of 40–50% due to root rot have been recorded in other areas (Erwin and Ribeiro, 1996). For example, in Vietnam they are higher by 15–20% due to inexperience in managing root and heart rot by farmers coupled with high ground water tables and the use of susceptible varieties. This is consistent with our results in which pineapple suckers were planted in buckets and watered regularly. Studies conducted by Drenth and Guest (2004) further re-enforces our yield loss findings, in their study in wet tropical areas of South Asia, the yield loss was estimated to be 6–12% a year due to extensive monsoonal wet periods which favors disease developments. Similarly, other crops have suffered from serious losses caused by the same or similar fungus, for example, black pod rot has been reported in almost all cocoa-producing countries, with worldwide losses estimated at 10% (Padwick, 1956). Direct crop losses of up to 90% occur in wetter areas such as Nigeria (Gregory and Maddison, 1981). A long-term field trial over a period of 10 years at Keravat in Papau New Guinea was reported with a mean pod loss of 17% (Holderness, 1992). The combination of different *Phytophthora* diseases of cocoa causes losses of 20–30% on the cocoa crop worldwide (Erwin and Ribeiro, 1996). According to Bowers *et al.* (2001), annual crop loss of 30–90% due to *Phytophthora* has been reported on cocoa and pineapple in Asia. In durian, *Phytophthora palmivora* which also causes losses in pineapple has been reported as a major cause of seedling dieback, leaf blight, root rot, trunk cankers, and pre-harvest and post-harvest fruit rots. Postharvest fruit rots result in10–25% losses of durian fruits (Lim, 1998).

According to Agrolink (2001) and Chau (1998), patch canker caused by *P. palmivora* is considered to be one of the major disease of durian in Malaysia while fruit rot caused by the same pathogen causes losses of 30% in durian and pineapple, respectively. In fact, it is estimated

that average disease losses and the cost of control of *P. palmivora* in durians and pineapple is in the range of 20–25% of production in Malaysia (Drenth and Guest, 2004).

Similarly, several diseases of rubber are attributed to a number of species of *Phytophthora,* including *P. botryosa, P. heveae, P.meadii, P. palmivora and P. nicotianae* (Erwin and Ribeiro 1996). Generally, diseases are responsible for losses of at least 10% of global food production, representing a threat to food security (Strange & Scott, 2005). Agrios (2005) estimated that annual losses by disease cost $220 billion and stated that to these, should be added 6–12% losses of crops after harvest, which are particularly high in developing tropical countries lacking infrastructure. Besides direct losses, the methods for disease control especially the chemical methods can result in environmental contamination and in residual chemicals in food, in addition to social and economic problems.

5.2 Conclusions

The study has shown that all the five pineapple cultivars used in this study were susceptible to pineapple heart rot disease. However, the field trial at Kayunga indicated that smooth Cayenne has moderate resistance to the same pathogen.

Yield loss assessment conducted at Kyambogo University screen house indicated that PHRD caused 100% yield loss where there was no protection.

5.3 Recommendations

Basing on findings and conclusions of these trials, the following are recommended:

i. An additional study needs to be conducted using more pineapple cultivars to establish their suitability as genetic material in breeding for resistance against PHRD.

ii. For now, farmers in areas where PHRD prevalence is high should consider growing Smooth cayenne which showed moderate resistance to PHRD.

iii. There is need for more evaluation on smooth cayenne to determine the mechanism of such resistance to PHRD, for detailed understanding of resistance to this pathogen.

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APPENDICES

Appendix 1. F. table for PHRD incidence, Trial 2 in an evaluation done in screen house, department of Agriculture, Kyambogo University, 2017

SOV	Df	INC 1	INC 2	INC 3	INC 4	INC 5	INC 6	INC 7	INC 8
REP	2								
VAR	4	12.533^{NS}	94.05^{*}	123.87^{NS}	396.56^{***}	231.86^{*}	115.81	131.19^{*}	2.353^{NS}
TRETMENT	2	17.622^{*}	919.72^{***}	3109.11***	9231.11^{***}	18118.49^{***}	30788.55^{***}	38605.16^{***}	49519.339^{***}
VAR+TRT	8	6.733^{NS}	76.05^{*}	125.97^{*}	365.29^{***}	205.55^{**}	95.67^{**}	111.16^{**}	1.957^{NS}

*** Significant at 0.001 ** Significant at 0.01 * Significant at 0.05 NS: Not Significant SOV=Source of variation VAR=Variety TRET= Treatments VAR+TRT =Variety and treatment interaction INC=Incidence in a week

Appendix 2. F. table for PHRD severity, Trial 2 in an evaluation done in the screen house, Department of Agriculture, Kyambogo University, 2017

SOV	Df	SEV 1	SEV 2	SEV 3	SEV 4	SEV 5	SEV 6	SEV 7	SEV 8
REP	2								
VAR	4	28.089^{*}	104.31^{*}	70.98^{NS}	322.64^{*}	48.89^{NS}	59.56^{NS}	90.59^{**}	18.47^{NS}
TREATMENT	2	96.200^{***}	1018.42^{***}	3305.40*	8050.87^{***}	22360.14^{***}	28168.16^{***}	35169.76^{***}	46722.64^{***}
VAR+TRT	8	21.089^{*}	71.98^{NS}	73.18^{NS}	312.58^{***}	39.10^{NS}	62.24^{NS}	63.59^{*}	15.35^{NS}

*** Significant at 0.001 ** Significant at 0.01 * Significant at 0.05 NS: Not Significant SOV=Source of variation VAR=Variety TRET= Treatments VAR+TRT =Variety and treatment interaction SEV=Severity in a week

Appendix 3. Disease incidence of PHRD, trial 2 in an evaluation done in the screen house, Department of Agriculture, Kyambogo University, 2017

VARIETY	INC 1	INC 2	INC 3	INC 4	INC 5	INC 6	INC 7	INC 8
MD-2 Hybrid	2.89	8.50	14.7	21.8	24.9	29.78	31.67	33.33
Red Spanish	0.89	8.67	11.0	21.2	25.6	31.67	34.33	34.39
Sasilimu	0.00	6.00	9.8	14.1	21.6	25.89	29.33	33.28
Smooth cayenne	0.00	0.67	4.4	5.5	13.0	22.50	24.00	33.11
Victoria	1.00	6.00	10.5	17.3	23.2	26.17	29.11	33.33
Mean	**0.96**	**5.97**	**10.1**	**16.0**	**21.6**	**27.20**	**29.69**	**33.49**
LSD(0.05)	**2.211**	**4.967**	**7.16**	**7.35**	**6.78**	**4.602**	**5.597**	**1.372**

INC 1= Incidence week 1

Appendix 4. Disease severity of PHRD, trial 2 in an evaluation done in the screen house, Department of Agriculture, Kyambogo University, 2017

VARIETY	SEV 1	SEV 2	SEV 3	SEV 4	SEV 5	SEV 6	SEV 7	SEV 8
MD-2 Hybrid	3.22	10.22	14.2	19.4	24.56	28.67	29.44	33.33
Red Spanish	3.33	7.33	11.1	20.2	25.56	29.00	32.78	33.67
Sasilimu	0.00	4.89	10.7	9.9	22.83	25.33	25.78	31.00
Smooth cayenne	0.00	1.11	6.4	7.3	19.89	23.56	24.78	33.28
Victoria	3.11	7.33	9.6	18.4	25.22	24.00	28.67	30.67
Mean	**1.93**	**6.18**	**10.4**	**15.1**	**23.61**	**26.11**	**28.29**	**32.39**
LSD (0.05)	**2.763**	**5.643**	**7.69**	**7.98**	**4.00**	**6.010**	**4.828**	**4.416**

SEV 1= Severity week 1

Appendix 5 .F. table for incidence severity and disease index of PHRD, season 2 in an evaluation at Kayunga, 2017

S.Variation	WK	2		4		6		8		10			12	12
	DF	I	S	I	S	I	S	I	S	I	S	I	S	DI
Rep	3													
VARIETY	4	24.165^{NS}	25.82^{*}	92.55^{NS}	107.8^{**}	155.9^{***}	184.1^{***}	221.0^{***}	200.5^{***}	163.4^{***}	155.1^{***}	177.5^{*}	79.66^{***}	42.74^{***}

***Significant at 0.001 **Significant at 0.01 *Significant at 0.05 NS: Not Significant

I= Incidence S= Severity DI= Disease Index

Appendix 6. F. table for PHRD incidence, trial 1 in an evaluation done in screen house, department of Agriculture, Kyambogo University, 2016

SOV	Df	INC 1	INC 2	INC 3	INC 4	INC 5	INC 6	INC 7	INC 8
REP	2								
VAR	4	2000.7^{***}	1489.3^{***}	1143.4^{*}	1105.1^{*}	1027.2^{NS}	998.8^{NS}	980.0^{NS}	893.3^{*}
TRET	2	38038^{***}	38038.0^{***}	417486.57^{***}	43365.1***	43660.6^{***}	43772.1^{***}	43846.7^{***}	44203.3^{***}
VAR+TRT	8	779.7^{NS}	653.7^{NS}	740.4^{NS}	798.4^{NS}	827.2^{*}	838.8^{*}	846.7^{*}	886.7^{*}

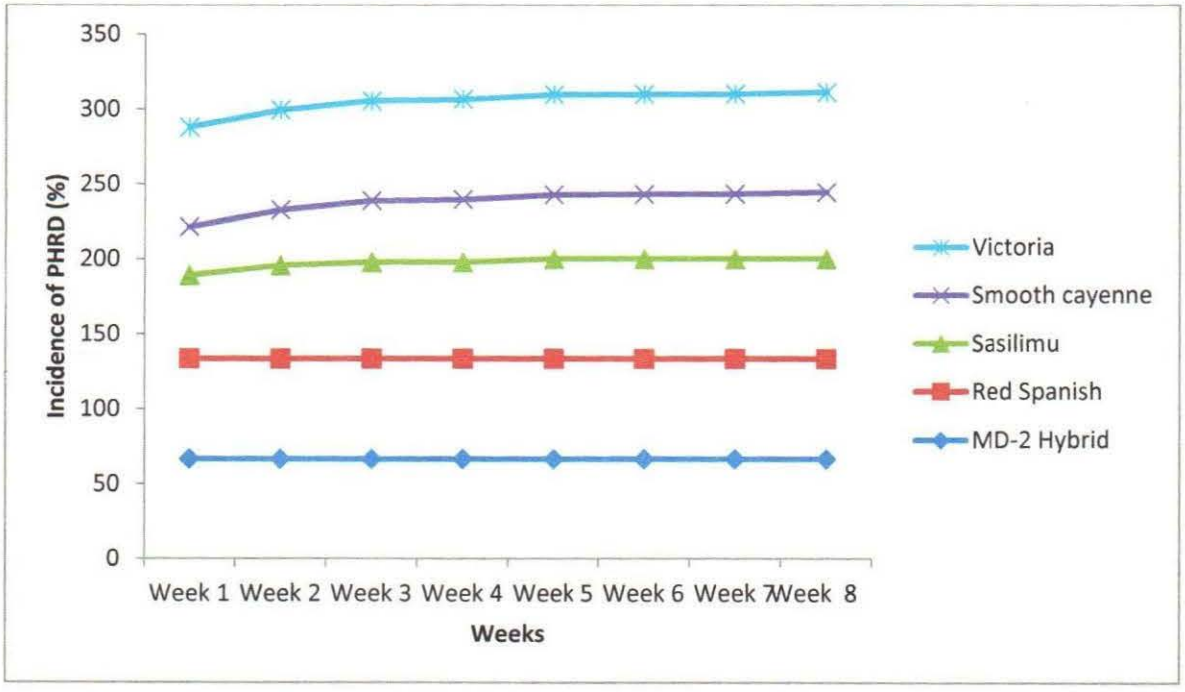
*** Significant at 0.001 ** Significant at 0.01 * Significant at 0.05 NS: Not Significant

Appendix 7. F. table for PHRD severity, trial 1 in an evaluation done in screen house, department of Agriculture, Kyambogo University, 2016

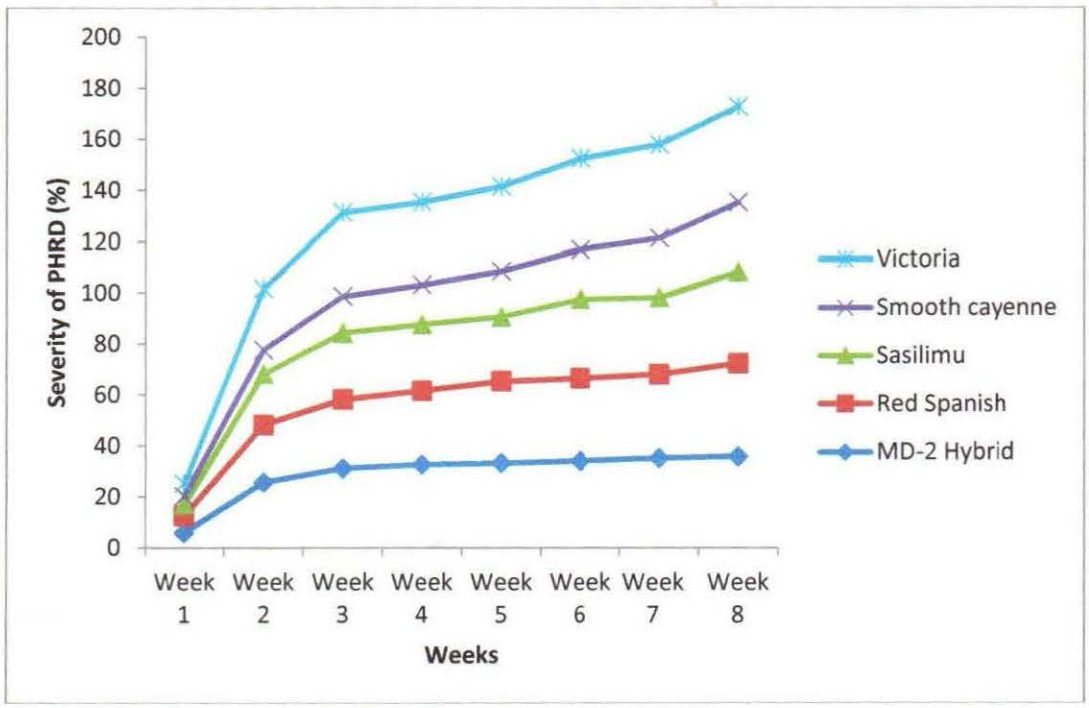
SOV	Df	SEV 1	SEV 2	SEV 3	SEV 4	SEV 5	SEV 6	SEV 7	SEV 8
REP	2								
VAR	4	14.797^{NS}	374.00^{***}	461.0^{NS}	440.91^{***}	401.94^{***}	381.87^{***}	255.32^{***}	5.70^{**}
TRETMENT	2	424.289^{***}	8011.27^{***}	14418.9***	440.91^{***}	401.94^{***}	381.87^{***}	255.32^{***}	42707.34^{***}
VAR+TRT	8	5.476^{NS}	184.10^{**}	236.8^{*}	198.65^{***}	206.75^{***}	151.88^{***}	98.59^{***}	2.21^{*}

*** Significant at 0.001 ** Significant at 0.01 * Significant at 0.05 NS: Not Significant SOV=Source of variation VAR=Variety TRET= Treatments VAR+TRT =Variety and treatment interaction SEV=Severity in a week

Appendix 8.Incidence of PHRD, trial 1 recorded in KYU screen house, 2016



Appendix 9. Severity of PHRD, trial 1 recorded in KYU screen house, 2016

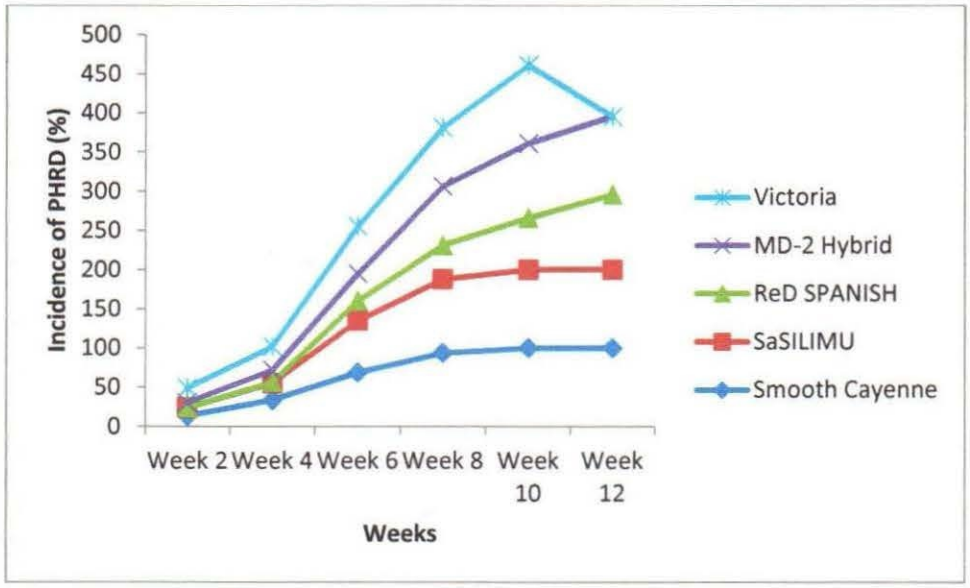


Appendix 10. F .table for incidence severity and disease index of PHRD, Season 1 in an evaluation at Kayunga, 2016

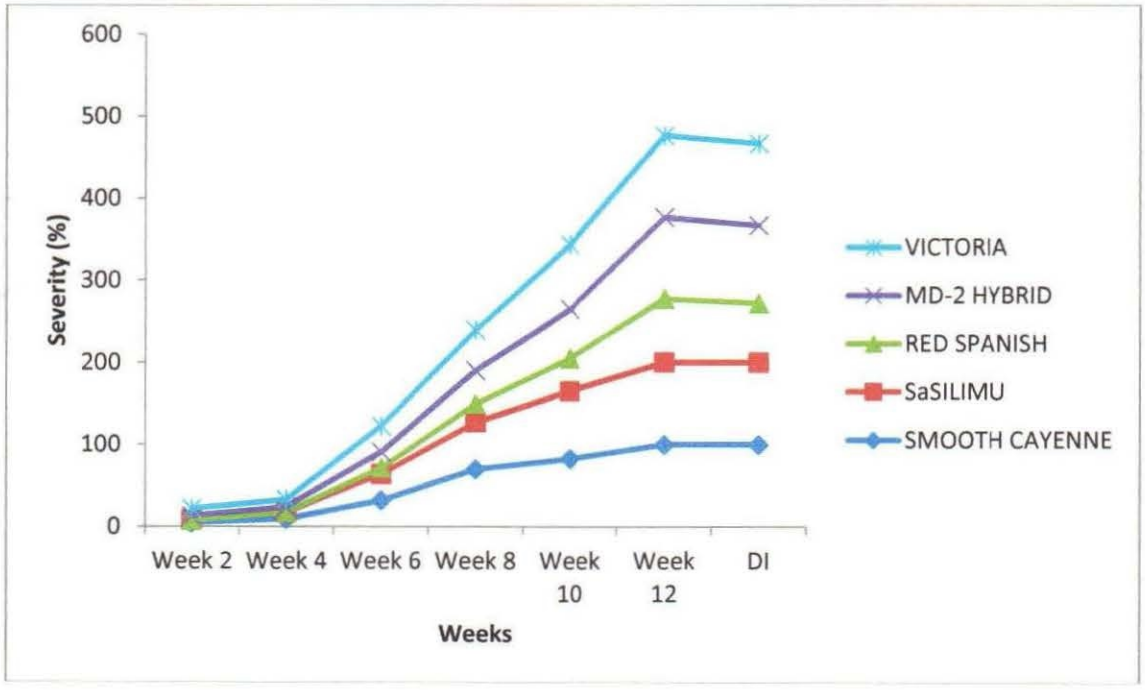
S.Variation	WK	2		4		6		8		10			12	12
	DF	I	S	I	S	I	S	I	S	I	S	I	S	DI
Rep	3													
VARIETY	4	204.4^{NS}	43.83^{NS}	673.8*	54.59*	1574.6**	473.5*	1671.88***	1272.5*	870.26***	1387.21***	14.925*	400.70*	580.6

***Significant at 0.001 **Significant at 0.01 *Significant at 0.05 NS: Not Significant

Appendix 11. Incidence of PHRD, Season 1 on five pineapple cultivars evaluated at Kayunga in May-2016.



Appendix 12 Severity and disease index of PHRD, Season 1 on 5 pineapple cultivar from week two up to week twelve in an evaluation at Kayung, 2016



DI=Disease index

Appendix 13 Genotypes of Pineapple from Uganda, Grown for PHRD resistance at experimental plot located in Kayunga in evaluation carried out in 2016.

Pineapple accession	Species
Red Spanish	*Ananas comosus* var. *comosus*
Smooth Cayenne	*Ananas comosus* var. *comosus*
Victoria	*Ananas comosus* var. *comosus*
Kasese hybrid	*Ananas comosus* var. *comosus*
Sasilimu	*Ananas comosus* var. *comosus*