

Reactions of pineapple cultivars to pineapple heart rot disease in central Uganda

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ABSTRACT

Pineapple (*Ananas comosus* (L.) Merr.) is an important fruit crop in Uganda ranking third as a major fruit crop after banana and citrus globally. However, pineapple production in Uganda is threatened by the recent outbreaks of pineapple heart rot disease (PHRD). Pineapple heart rot disease is the most widespread and devastating disease of pineapple in Uganda and can cause tremendous yield loss. Information on the sources of resistance is still scanty in Uganda. Additionally, no study has been done in Uganda which point out whether variety influences disease severity. Therefore, the objective of this study was to assess the reaction of pineapple cultivars to pineapple heart rot disease in central Uganda. Five cultivars of healthy pineapple suckers were planted in the field in a randomized complete block design (RCBD) to screen for resistance to PHRD. No significant ($p > 0.05$) difference in PHRD incidence was recorded in week two and week 4 of the experiment. However, there was significant difference ($p < 0.001$) in incidence of PHRD from week 6 up to week 12 of the experiment. Additionally, PHRD severity varied significantly ($p < 0.001$) from week 6 up to week 12. Cultivar Smooth Cayenne recorded low severity and is therefore promising. All the five pineapple cultivars were susceptible to PHRD though with varying levels of incidence and severity. Therefore, there is need for additional studies to establish the suitability of Smooth Cayenne as a source of parental donor in breeding for resistance to PHRD.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr) is one of the most important plants in the family Bromeliaceae, (Bartholomew 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 Uganda, pineapple is widely grown in the central region in the districts of Mukono, Kayunga, Luwero and Masaka (Bua et al., 2013). In fact, Pineapple is one of the crops in the fruit sub sector that has been selected for export diversification and sustainable enhancement of household incomes in Uganda (UIA, 2015). As a result, many farmers within Mukono, Kayung, Luwero and Masaka have taken on pineapple production. According to Kwikiriza et al. (2016), 75% of pineapple in Uganda was exported in 2013/14 out of the 400,000 metric tons produced in the same year. However, pineapple production is still below optimum because of a diversity of constraints (UIA, 2008). Pests and diseases, lack of improved pineapple production technologies and declining soil fertility are among the notable constraints (Bua et al., 2013). The genus *Phytophthora*, the causal organism of PHRD is a very

destructive plant pathogen that attacks hundreds of plant species including pineapple throughout the world (Drenth and Guest, 2004). However, the most common species of *Phytophthora* causing severe damages to pineapple in Uganda has been identified as *P. nicotianae*. PHRD has been reported and confirmed in all the major pineapple production regions of the country (Ocwa et al., 2017a,b). 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). Unless well managed, PHRD can cause 100% yield loss. Accordingly, resistance has been advocated as the most economical and cost-effective methods for controlling a number of plant diseases (Drenth and Guest, 2004; Agrios, 2005). In fact, *Bromelia pinguin*, was reported as one of the cultivars of pineapple that is resistant to *Phytophthora nicotianae* var. *parasitica* compared to varieties like Serrana, Red Spanish Enana, Spanish Nozera'n Red, Spanish Colorada Caney, Red Spanish Pinareña, Red Spanish Colorada Ramo'n which were all susceptible (Rodríguez et al. 2002). Therefore, the basis of this study

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was to assess the reaction of pineapple cultivars to PHRD in central Uganda.

2. Materials and methods

2.1. Study area

This experiment was conducted in Nazigo sub-county, Kayunga district, Uganda from May 2016 to September 2016 and repeated during the period October 2016 to March 2017. Kayunga district is located approximately 74 km (46 mi) northeast of Kampala, on an all-weather road. The experimental field was located in the area with coordinates 00° 35.245N, 033° 02.251E with elevation of 1106 metres above sea level. The experiment was set in a field which has been under pineapple production for four years with high pineapple heart rot disease incidence. The disease incidence was ascertained as described by Bua *et al.* (2013). Nazigo is characterized by heavy clay loam, a characteristic of the Lake Victoria crescent. The mean maximum and minimum temperature ranged from 27–30 °C and 15–18 °C, respectively. The area is also characterized by bimodal type of rainfall with the mean annual rainfall of 1000 mm with the peaks in May and October (Bolwig, 2012; Raphael *et al.*, 2016).

2.2. Collection of planting materials

Disease free pineapple suckers of the five cultivars including Smooth Cayenne, Victoria, MD-2 hybrid, Red Spanish and *Sasilimu* were purposively collected from the districts of Masaka, Luwero, Mukono and Kayunga. The disease-free status of the suckers was confirmed by planting the suckers in the pots filled with sterilized soils in the screen house for two months from November to December 2015. 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 used for the field trial in Nazigo sub-county, Kayunga district.

2.2.1. Inoculum quantification

Inoculum was quantified by collecting twenty soil samples (200 g each) diagonally from the experimental plot. Soil samples were packed in paper bags and taken to the laboratory. Samples were air dried for four days. All the twenty samples (4000 g) were mixed together and a representative sample of 500 g taken. 1000 ml of distilled water was used to dissolve the 500 g of soil to make a non-sterile soil extract (NSSES) solution (Rodriguez *et al.* 2015). Zoospore release was induced by incubating non-sterile soil extract solution at 4 °C for 30 minutes. The solution was later placed at room temperature for 10–20 minutes to burst the sporangia so as to release zoospores (Saadoun and Allagui, 2008). Zoospores density was determined using the method described by Rodriguez *et al.* (2015). Uniformity of inoculum density in the experimental field was further improved by ring application of 10 g of infected soil on each hole of pineapple plant at planting (Kuniaki *et al.*, 2016).

2.3. Experimental set up/layout

The experimental design was a randomized complete block design (RCBD) replicated four times. Each replicate block comprised of 100 pineapple plants in double rows. Each plot measuring 35 metres × 10 metres represented a replicate with treatments. The plots within the block were separated by an alley of 1 metre while the block by 2 metres (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 0.45 metres × 0.3 metres on 26th May 2016 and 1st October 2016, respectively in hotspot area of PHRD.

2.4. Field preparation

The experimental plots were deep ploughed and harrowed to obtain the right tilt. Each replicate bed was mulched with *Paspalum* species grass after planting to help retain moisture in the soil and to control weed growth on the beds. All normal agronomic practices for pineapple cultivation were followed (Nakasone and Paull, 1998).

2.5. Data collection

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 incidence was determined as the number of plants diseased expressed as a percent of the total number of plants assessed per replication. Disease severity was assessed on individual 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 (De Sousa *et al.*, 2013; Rohrbach and Schenck, 1985).

Disease index (DI) was assessed 90 days after planting using a formula;

$$\sum (DI \times \text{number of plants showing symptoms} / \text{total number of plants observed})$$

where disease index (DI) refers to the belowground part of the plant (Kuniaki *et al.*, 2016). In each sample unit, the suckers were pulled out from the soil and the belowground part assessed for PHRD symptoms (Kuniaki *et al.*, 2016). Cultivars in which infection took place but the colonization of the host tissue proceeded slowly, reaching a disease index of 41–60% or less 3 months after natural infection and which showed significantly less disease severity were considered as resistant to PHRD (Rohrbach and Schmitt, 1994; Kuniaki *et al.*, 2016).

2.6. Data analysis

Data on incidence, severity and disease index were summarized in Micro Soft excel 2008 edition, and means subjected to analysis of variance (ANOVA) using Genstat (15th edition). Where there were significant differences, means were separated using Least Significant Difference (LSD) test at 5% probability level.

3. Results

All the five cultivars were susceptible to PHRD though with varying levels of incidence. However, twelve weeks after planting, MD-2 hybrid, Red Spanish and Victoria recorded 100% incidence (Fig. 1). Similarly, severity among the cultivars followed a similar trend as in the incidence (Fig. 2). Additionally, disease index score during the twelfth week of season one was 100% on MD-2 hybrid, Red Spanish and Victoria. Smooth Cayenne and *Sasilimu*, recorded less than 100% disease index (Fig. 3). No significant difference in PHRD incidence and severity was recorded from week two of the experiment ($p > 0.05$). However, there was significant difference ($p < 0.001$) in incidence and severity among the cultivars from week four up to week twelve. Additionally, the highest and lowest incidence of PHRD two weeks after planting in season two trials was recorded on MD-2 hybrid and Smooth Cayenne, respectively (Fig. 3). A similar trend was followed during the twelfth week (Fig. 4). Correspondingly, the highest and lowest PHRD severity in season 2 trial was recorded on MD-2 hybrid and Smooth Cayenne, respectively (Fig. 5). Disease index score in season two followed a similar trend recorded in season one (Fig. 6). Overall, no significant ($p > 0.05$) differences in PHRD incidence were recorded in early stages of the experiment. However, there were significant differences ($p < 0.001$) in incidence and severity of PHRD from week six up to week twelve of the experiment.

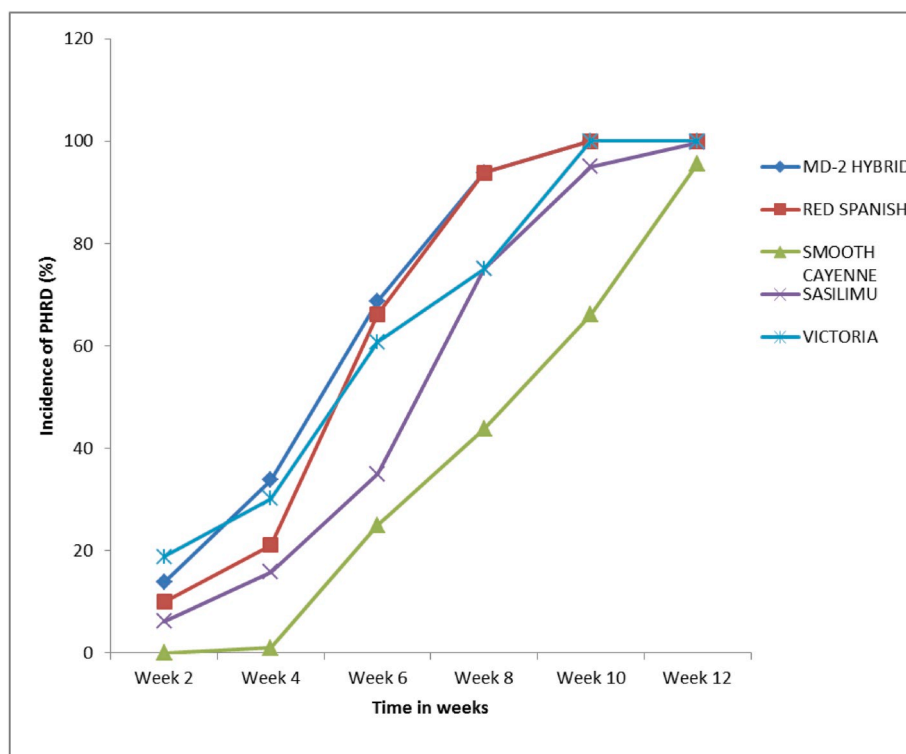


Fig. 1. Incidence of PHRD, Season 1 on five pineapple cultivars evaluated at Kayunga, 2016.

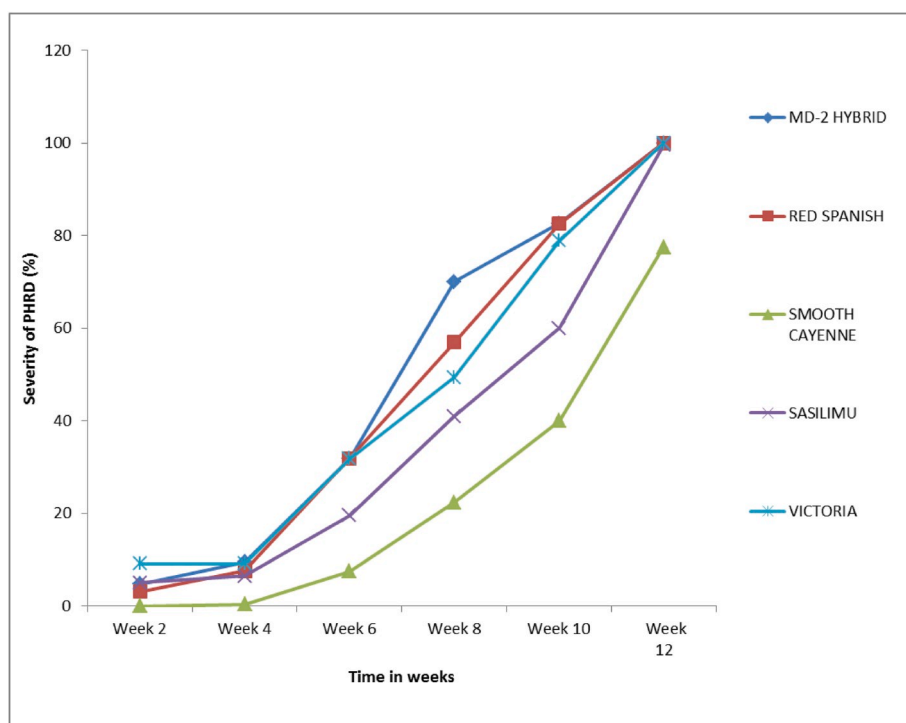


Fig. 2. Severity of PHRD, Season 1 on five pineapple cultivars evaluated at Kayunga, 2016.

4. Discussion

The results indicated that all the cultivars were susceptible to PHRD. This could be as a result of the genetic composition of the cultivars with limited resistant genes against the PHRD pathogen. Additionally, the quantity of the inocula in the experimental site could have resulted in

the high level of severity. This is particularly possible since the site had history of severe PHRD. This compares very well with the findings of [Rodriguez 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

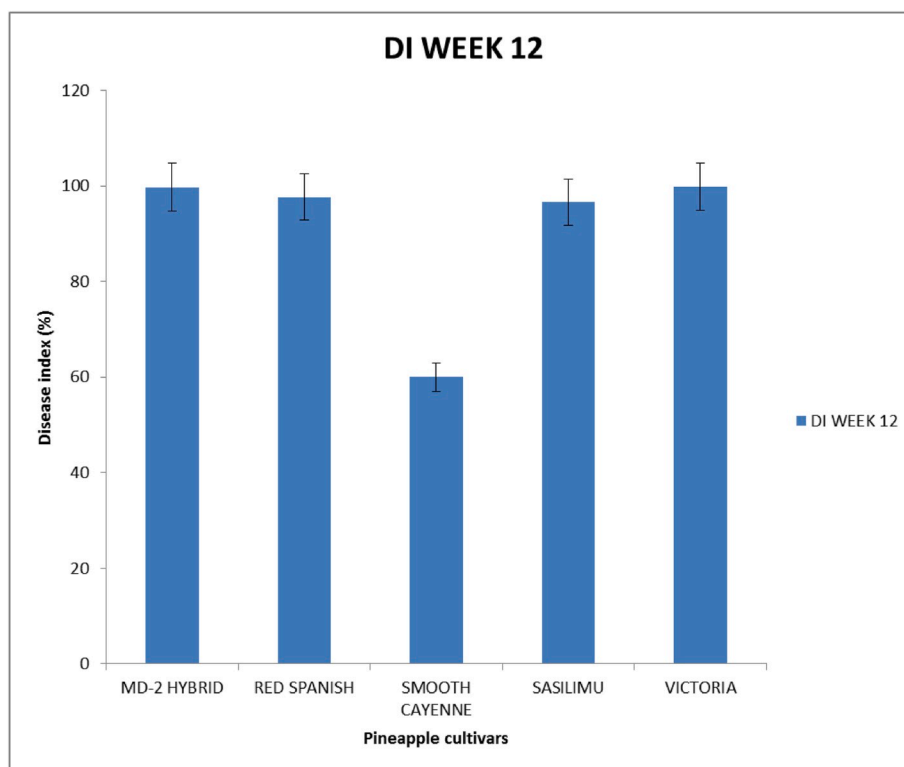


Fig. 3. Disease index of PHRD, Season 1 on five pineapple cultivars evaluated at Kayunga, 2016.

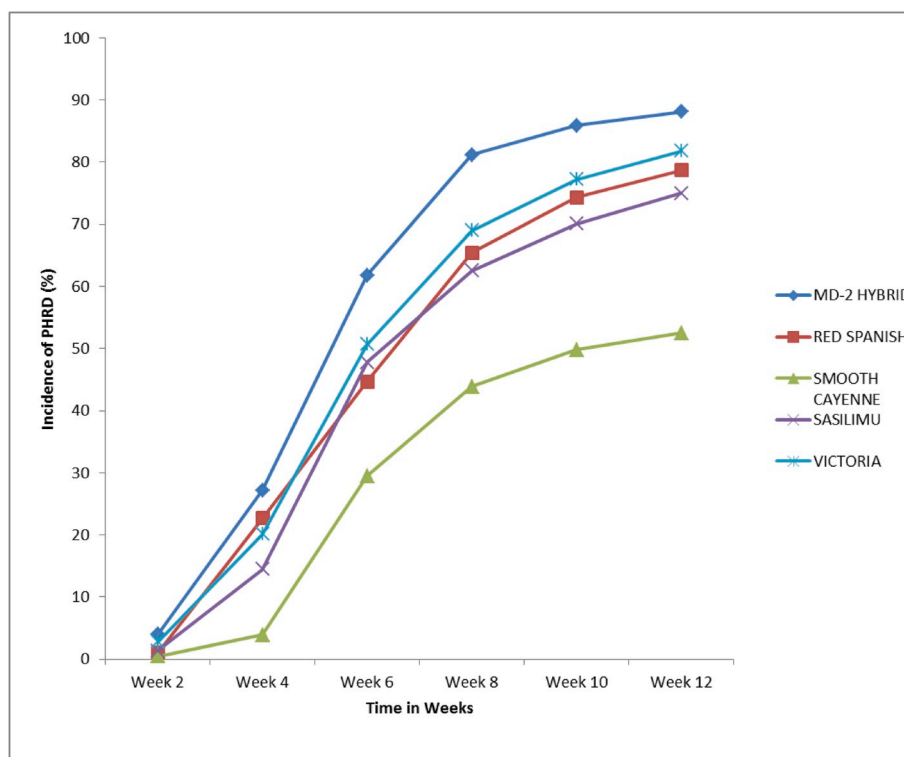


Fig. 4. Mean incidence of PHRD, Season 2 on five pineapple cultivars evaluated at Kayunga, 2017.

same disease.

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 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 in a monoculture system have contributed to the severity of several pests and diseases including PHRD. In the same study,

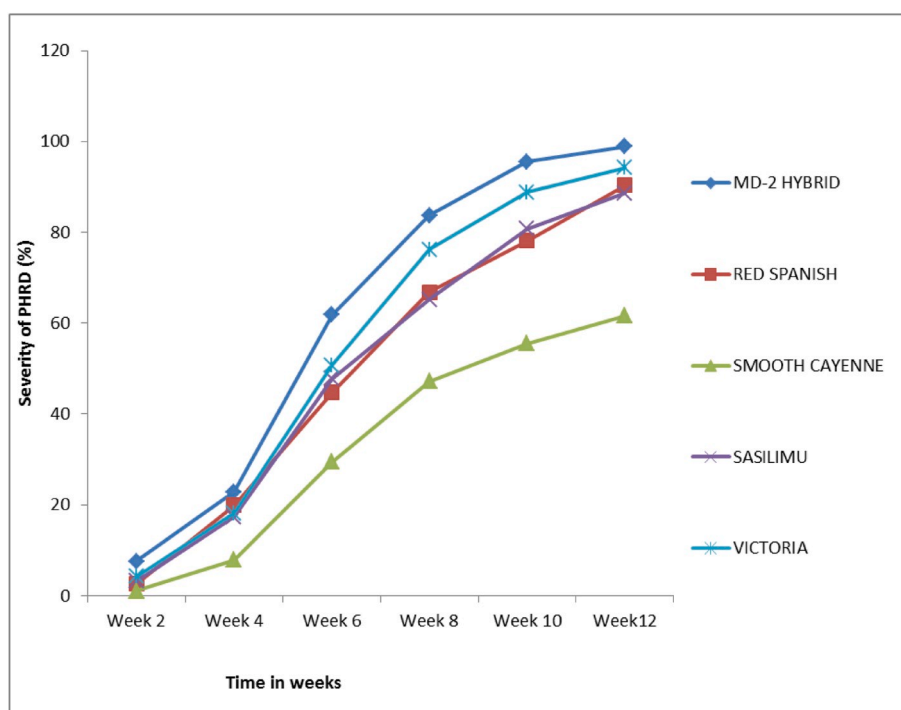


Fig. 5. Mean severity Of PHRD, Season 2 in an evaluation at Kayunga, 2017.

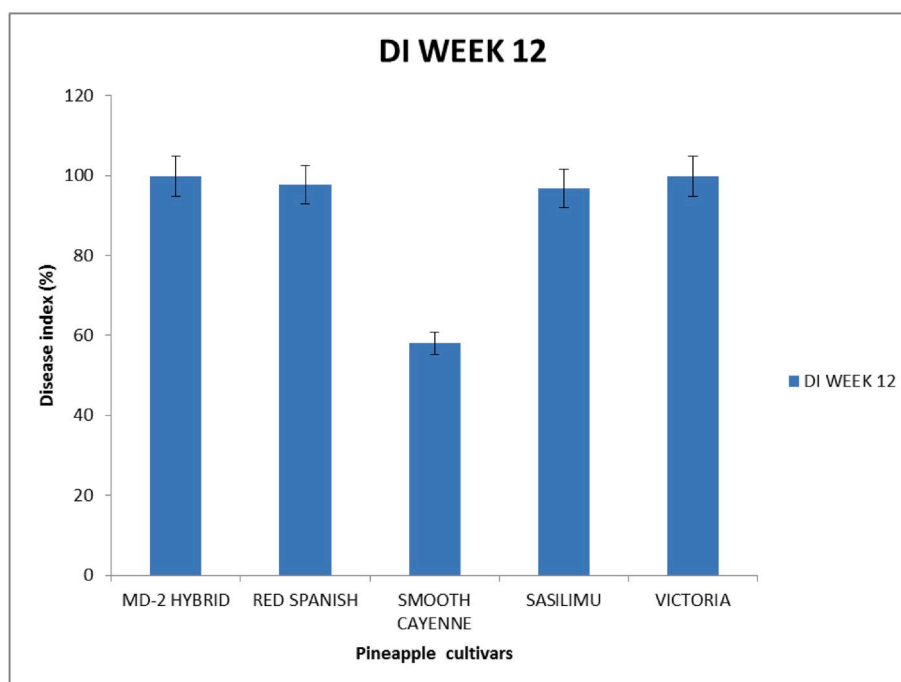


Fig. 6. Disease index of PHRD, Season 1 on 5 pineapple cultivar, week twelve in an evaluation at Kayung, 2016.

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. Relatedly, Rohrbach & Schmitt (1994) reported in their findings that cultivar smooth Cayenne is resistant to diseases like mealy bug wilt, *fusariosis*, fruitlet core rot, butt rot, internal browning and PHRD which is consistent with the result of this study.

In spite of the high level of susceptibility recorded in this field trial, disease severity in cultivar Smooth Cayenne was relatively slow. This 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, accumulate starch reserves, thus becoming vigorous compared to other cultivars (Bartholomew et al. 2003). Furthermore climatic conditions determine active growth of the plant and hence susceptibility to both biotic and a biotic stress (Bartholomew et al. 2003). Thus, it can be postulated that the weather condition in the experimental site was more favorable to the pathogen and less favorable to the cultivars evaluated in this trial. This relates very well to the findings of James and Scot (2015) who

demonstrated that smooth Cayenne is highly resistant to PHRD when environmental conditions do not favor the pathogen. Additionally, Py et al. (1987) and Lim (1985) also noted that cultivar smooth Cayenne is resistant to fruit collapse caused by *Erwinia chrysanthemi* and *Phytophthora* sp under favorable environmental conditions. This concretizes the results of this study.

Relatedly, Bartholomew et al. (2003) pointed that it is the practice in commercial cultivation of pineapple to plant one cultivar in a monoculture system. In fact, in Kayunga, only one cultivar of pineapple smooth Cayenne locally known as *Nalongo* is almost exclusively cultivated. The other cultivars are almost not present in the pineapple agricultural ecosystems in Kayunga. Therefore, intraspecific gene transfer can potentially only occur within this cultivar and its clones (Bartholomew et al., 2003). This perhaps may partly explain why smooth Cayenne, a dominant cultivar in the study location comparatively recorded low disease incidence, severity and disease index in this experiment. Also, the fungus that causes PHRD is a slow growing pathogen; in addition, this fungus has 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 into slow disease progress recorded in this study. This is in agreement with study conducted by Chakraborty et al. (1991) who demonstrated that the effectiveness of resistance levels depends not only on the availability of resistance genes but also on the nature and speed of the life cycles of the pathogens as well as their means of spread. Similarly, the effects of zoospore concentration in the experimental field could have influenced disease severity among the pineapple genotype used in this study. This study therefore confirms findings from Rodriguez et al. (2002) which 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.

5. Conclusion and recommendation

The result of this study has shown that all the five pineapple cultivars were susceptible to PHRD though with varying levels of incidence and severity. Therefore, there is need for additional studies to establish the suitability of smooth Cayenne as a source of parental donor in breeding for resistance to PHRD.

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Declaration of competing interest

The author(s) have declared no conflict of interest.

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