

Research Application Summary

Pathogenicity of pineapple heart rot disease causal organisms in Central Uganda

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Abstract

Pineapple (*Ananas comosus* L. Merrill) is one of the most important horticultural crops in many tropical and sub-tropical countries including Uganda. Currently, pineapple production in Uganda is being threatened by the outbreak of Pineapple heart rot disease (PHRD). Information on the pathogenicity of the causal organisms in Uganda is still scanty. Therefore, this study determined the pathogenicity of pineapple heart rot disease causal organisms from central Uganda. Pathogenicity of the pineapple heart rot causal organisms was assayed on green apple fruits and pineapple plants in the laboratory and the screen house. Results indicated that out of the seven pure cultures used for inoculation, five were pathogenic. Ninety five percent (95%) of all inoculated plants developed heart rot disease symptoms and characteristics with the emergence being observed within 4-7 days and 9-20 days on green apples and pineapple plants, respectively. Therefore this study has proved that PHRD causal organisms in Uganda affect pineapple and the effect manifests within a short period of time.

Key words: Causal organisms, heart rot, pathogenicity, Phytophthora isolates

Résumé

L'ananas (*Ananas comosus* L. Merrill) est l'une des cultures horticoles les plus importantes dans de nombreux pays tropicaux et sous-tropicaux, y compris l'Ouganda. À l'heure actuelle, la production d'ananas en Ouganda est menacée par la pathologie de la pourriture du cœur de l'ananas. L'information sur la pathogénicité des organismes responsables en Ouganda est encore insuffisante. Par conséquent, cette étude a évalué la pathogénicité des organismes responsables de la pathologie au centre de l'Ouganda. La pathogénicité a été testée sur des fruits à la pomme verte et des plantes d'ananas dans le laboratoire. Les résultats ont indiqué que sur les sept cultures pures utilisées pour l'inoculation, cinq étaient pathogènes. Quarante-vingt-cinq pour cent (95%) de toutes les plantes inoculées ont développé des symptômes et caractéristiques de la pathologie dans une période de 4 à 7 jours et 9 à 20 jours sur les pommes vertes et les ananas, respectivement. Par conséquent, cette étude a prouvé que les organismes responsables de la pathologie en Ouganda affectent l'ananas et les effets se manifestent dans un court laps de temps.

Mots clés: Organismes responsables, pourriture cardiaque, pathogénicité, isolats de *Phytophthora*

Background

Pineapple (*Ananas comosus* L. Merrill) is an important horticultural crop in many tropical and sub-tropical countries including Uganda (Sonko *et al.*, 2005; Akhilome *et al.*, 2015). Pineapple production on a global scale is being constrained by abiotic and biotic stresses such as drought, soil infertility, pests and diseases, among others (Kuwornu *et al.*, 2013; Akhilome *et al.*, 2015). Studies (NARO, 2012; Bua *et al.*, 2013) around the Lake Victoria crescent indicate that pineapple is under threat from the outbreak of diseases especially pineapple mealy bug wilt disease (PMWD) and pineapple heart rot disease (PHRD). This challenge is not unique to the Lake Victoria crescent as elsewhere, pineapple heart rot disease is widespread and devastating. For example 25%-30% prevalence has been observed in China (Shen *et al.*, 2013). With the devastating potential of complete yield loss (Rohrbach and Schenck, 1985; Green and Scot, 2015) and the rapid spatial and temporal spread of the disease (Joy and Sindhu, 2012), pineapple heart rot disease threatens the food security, livelihoods and pineapple biodiversity in Uganda.

Pineapple heart rot disease manifests as the base of the heart leaves become water soaked causing foul smell, heart leaves wilting, browning and easily pulling off (Shen *et al.*, 2013; Green and Scot, 2015). Several researchers (Joy and Sindhu, 2012; Shen *et al.*, 2013; Rodriguez *et al.*, 2015) have reported that pineapple heart rot disease is caused by a wide range of *Phytophthora* species. There is however limited knowledge of which species are menacing Uganda at present (NARO, 2012; Bua *et al.*, 2013). Therefore, the objective of this study was to determine the pathogenicity of pineapple heart rot disease causal organisms from Uganda.

Literature summary

Pineapple heart rot has many causal species including the *Phytophthora* that has consistently been isolated from infected pineapple plants worldwide and these are *P. cinnamomi*, and *P. nicotianae* (Joy and Sindhu, 2012; Shen *et al.*, 2013; Green and Scot, 2015; Rodriguez *et al.*, 2015). However, there is variation in the manifestation of PHRD depending on the variety of pineapple (Rodriguez *et al.*, 2002). Pineapple heart rot disease can cause up to 100% yield loss (Rohrbach and Schenke, 1985). The manifestation is further complicated by the great diversity, high aggressiveness and the multicyclic nature of *Phytophthora* (Milenkovic *et al.*, 2014). It is this aggressiveness that causes systemic foliar necrosis (Cecile and Guests, 1994; Rodriguez *et al.*, 2015). For instance, the development of the distinctive hard brown rots on the apples is an indication of the aggressiveness of the causal organisms (Serfontein *et al.*, 2007). As such, pathogenicity information becomes important in determining the host specificity and variation in virulence among isolates of *Phytophthora* (Robin and Desprez-Loustau, 1998).

Study description

The study was conducted in 2015 at the National Crops Resources Research Institute, Namulonge (NaCRRI) and Kyambogo University laboratories.

Eighty symptomatic pineapple samples collected from the four major pineapple growing districts of central Uganda including Masaka, Luwero, Mukono and Kayunga were used in the study. Pathogen isolation and culturing was done using Cornmeal Agar amended with Pimaricin, Ampicillin, Rifampicin, Benomyl, Pentachloronitrobenzene and Hymexazol (PARBPH) as described by Drenth and Sendall (2001). Diseased tissue fragments 2-4mm wide were cut from the edge of the lesions and disinfected by dipping in 70% ethanol for three minutes, rinsed in sterilized distilled water (SDW), blot dried and plated on Cornmeal Agar amended with 10mgs Pimaricin, 250mgs Ampicillin, 10mgs Rifampicin, 10mgs Benomyl, 25mgs Pentachloronitrobenzene, and 50mgs of Hymexazol (PARBPH). Inoculated plates with media were incubated at 24°C in the dark and examined within 2–3 days (Drenth and Sendall, 2001; Mbaka *et al.*, 2009; Mounde *et al.*, 2012). Pure cultures were obtained by sub culturing hyphal tips onto a freshly prepared and amended corn meal agar (ACMA).

Pathogenicity of the pure cultures was conducted following the procedures of Mbaka *et al.* (2010) and Sadeghy *et al.* (2014) with a modification. Green apple fruits surfaces were sterilized with 70% alcohol and blot dried. Incisions were cut into the apples using a sterile scalpel and agar plugs of *Phytophthora* cultures of 5mm diameter were inserted with mycelia facing downwards. The points of inoculation were sealed with parafilm. Controls were inoculated with agar discs without pathogen and sealed in the manner described above. There were three replicate apples for each isolate and control experiments. The pineapple heart leaves were inflicted with four lesions using sterile needle (Rodriguez *et al.*, 2015) and inoculated with a *Phytophthora* isolates suspension with mycelium and sporangium (Sadeghy *et al.*, 2014).

Results

Results showed that five out of seven isolates recovered were pathogenic. Symptoms were observed between 4-7 days and 9-20 days on green apples and pineapple plants, respectively (Figure 1 and Table 1). This finding therefore compare very well with that of Mbaka *et al.* (2010) who observed a hard brown rot on green apples within one week of inoculation. According to Rodriguez *et al.* (2015) symptoms of PHRD on the inoculated pineapple plants often show up with 7-20 days after inoculation. The manifestation of PHRD is gradual as indicated by a pale green color of heart leaves, browning of the base of the middle leaves and heart rot coupled with foul smell. Although the samples used in this study were few, it is evident that isolates were pathogenic hence they can be used for screening pineapple germplasm for resistance to the disease since Thomidis *et al.* (2002) noted that host susceptibility has implication to disease management. More importantly, this result has provided the first

comprehensive information on the pathogenicity of pineapple heart rot disease causal organisms in Uganda. However, additional studies are underway to identify the morphological characteristics of the isolates as well as assess their *invitro* reaction to the commonly used antifungals.

Additionally, the apple pathogenicity tests used in this study were accurate in separating pathogenic and non pathogenic *Phytophthora* recovered from infected plant parts (pineapple leaves). This can be used to determine levels of *Phytophthora* inoculum in soils and evaluating efficacy of soil treatments like drenching with chemicals for control of PHRD (Mbaka *et al.*, 2010). However, Stewart (1991) noted that pathogenicity of isolates determined under controlled glasshouse conditions may not reflect the overall pathogenicity of a population of the species within a field soil hence the need to replicate this study under field conditions. This is because pathogenicity tests provide a basis for clarification of the role of the causal organism(s) in decline in the productivity of the host plant (Milenkovic *et al.*, 2014) which is indicated by the presence of visible disease symptoms on a host plant in absence of latent infection (Mbaka *et al.*, 2010).

The symptoms observed on the inoculated pineapple plants in this study were similar to those observed during the field survey (Rodriguez *et al.*, 2015). Considerably high number (95%) of the inoculated pineapple plants produced PHRD typical symptoms within three weeks compared to the inoculated control (Figure 1). This means that the causal organisms in the isolates can survive under varying conditions in the laboratory and screen house. This is consistent with Thomidis *et al.* (2002) who observed that results from laboratory and glasshouse experiments using apples were correlated.

Table 1. Reaction of apples and pineapple plants to *Phytophthora* isolates in central Uganda, 2015.

Isolate number	Location	Response after inoculation	
		Apples	Pineapple plants
001	Kayunga	+	+
002	Kayunga	+	+
003	Kayunga	+	+
006	Kayunga	-	-
001	Luwero	+	+
002	Luwero	+	+
003	Luwero	-	-

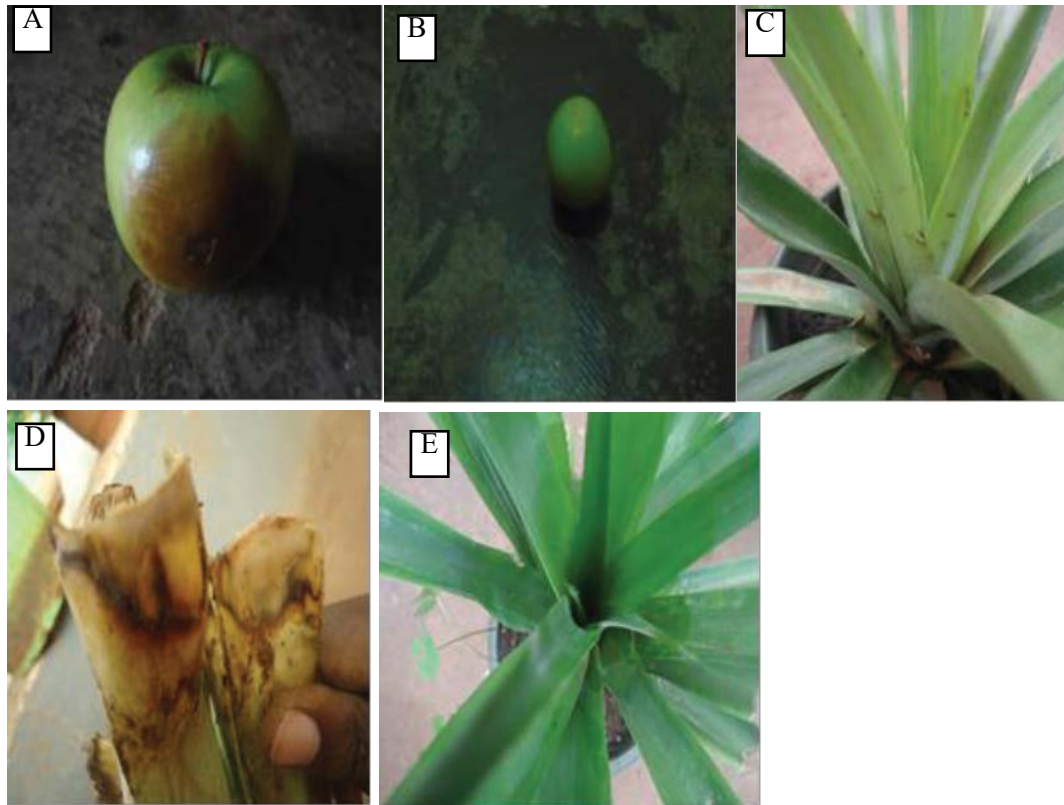


Figure 1. Symptoms of heart rot disease on green apples and pineapple plants in central Uganda, 2015. A) Apple showing a hard brown rot of pineapple heart rot disease 4 days after inoculation. B) Control apple C). Pineapple plant showing pale green colour of pineapple heart rot disease 9 days after inoculation. D) Necrotic heart leaves of inoculated pineapple plant with a foul smell extracted from inoculated pineapple plant 3 weeks after inoculation. E). Control pineapple plant.

Recommendation

Pineapple heart rot disease causal organisms in Uganda are highly pathogenic and devastating. Therefore there is need to conduct a detailed study on the identity of causal organisms as well as assess the management options to pineapple heart rot disease.

Acknowledgement

Funding for this study was provided by the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) Grant Number – RU 2014 GRG- 085 awarded to the second author. This paper is a contribution to the 2016 Fifth African Higher Education Week and RUFORUM Biennial Conference.

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