FRUIT, LEAF AND STEM DISEASES OF *CARICA PAPAYA* L

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Abstract

Control of C. papaya diseases requires intervention both on the field and post harvest. Leaf and stem diseases may spread to the fruit and result in poor fruit yields. This study described common papaya diseases of the fruit, leaf and stem with associated pathogens of C. papaya L. Powdery mildew affecting fruit, leaf and stem, necrotic stem, Phytophthora blight, stem end rot and Erwinia rots of fruits characterized field diseases while anthracnose, watery soft rot and Alternaria spots were frequent post harvest fruit rots. The primary pathogens were moulds (Alternaria, Rhizopus, Phomopsis, Phytophthora, Oidium and Colletotrichum) and a bacterium, Erwinia. The other bacteria genera isolated (Klebsiella, Enterobacter, Pseudomonas, Bacillus, Streptococcus and Gram positive short rods) were more of secondary invaders are importantly human pathogens and thus of health significance. Post-harvest damage of C. papaya fruit is influenced by the quality of harvested fruit, an incubating disease and storage conditions.

Key words: *carica papaya, disease incidence, disease severity, fungal agents, disease vectors*

1. INTRODUCTION

Nigeria is listed among the top five countries cultivating *Carica papaya* L. (Bautista-Baños et al. 2013), though majority of its fruit is consumed locally. The absence of pawpaw (papaya) processing industry in Nigeria does not justify the large scale farming in the crop. The fruit is consumed fresh and this has not translated into economic gains for farmers who on several occasions suffer losses due to microbial attack, poor handling of fruits and lack of adequate storage facility (Singh, 2010; Paull et al. 1997). Fungi are predominantly associated with *C. papaya* diseases and their effect may be so devastating that an entire orchard may be affected. Koffi et al. (2010) reported significant losses in papaya orchards in Côte d'Ivoire due to *Pythium aphanidermatum*. This fungus which was the primary pathogen predisposed the plants to secondary infections with *Fusarium* and *Rhizoctonia* species. Cunningham and Nelson (2012) described powdery mildew caused by *Oidium caricae-papayae* as a threat to *C. papaya* orchards in Hawai'i. Other major fungal pathology of *Carica papaya* across the globe is Phytophthora blight and anthracnose (Nelson, 2008; Aires et al. 2004; Casarrubias-carrillo et al. 2002). The papaya ringspot virus (PRSV) and papaya meleira virus have been reported as the major virus causing significant economic losses to *C. papaya* farming (Rodrigues et al. 2009; Diallo et al. 2008; Ventura et al. 2003).

Though major concern has been on post-harvest deterioration of *C. papaya* fruits, attention is now being focused on infections occurring in the fields as these invariably results in fruit quality highly susceptible to microbial attack or fruits that are not marketable (Spotts et al. 1999; Sugar et al. 1992). In other to reduce losses in *C. papaya* fruit harvest, an understanding of the pathogenesis of diseases of the plant especially those caused by fungi is paramount. Bhale (2011) indicated that *C. papaya* fungal fruit infections may occur at any of these stages; during the growing season, harvesting, handling, transport and post-harvest storage and marketing conditions. Also the rich nutrient content and low pH generally adapt fruits to microbial degradation (Singh and Sharma, 2007). More organisms have been identified with post-harvest rot of papaya fruits than fruits on the tree. This can be accounted for by the decrease in defensive mechanisms once the fruit is detached off the tree. It is important to monitor plant performance in a field as this will inform farm management strategies for improve yield. The present study was carried out following increase in fruit rots in our demonstration farm 2-3 years after cultivation. This report is an overview of disease incidence and aetiology in a university model research farm.

2. MATERIALS AND METHODS

2.1. Assessment of disease occurrence and severity

A systematic field survey of *C. papaya* rot diseases was carried out on the farm from 2011 to 2014. Disease incidence (DI) was calculated using Cooke's equation (Cooke, 2006) while the disease severity (DS) was determined applying the rating scale of Bowen (2004) in which 0 = no symptom, 1 = 0-20 % severity level on infected plants, 2 = 20-40 %, 3 = 40-60 %, 4 = 60-80 % and 5 = 80-100 % and determined using the equation of Kranz (1988) as given below:

$$DI\ (\%) = x/N \times 100$$

$$DS\ (\%) = \sum(a+b)/N.Z \times 100$$

$$\text{i.e. } \frac{\sum(\text{value of grade} \times \text{number of fruits or leaves or stems with spots or infections})}{\text{Number of any part examined} \times \text{the maximum value of score scale (grade)}} \times 100$$

Where; $\sum (a+b)$ = Sum of infected fruits and their corresponding score scale

i.e. $\sum$ (value of grade × number of fruits or leaves or stems with spots or infections)

N = Total number of sampled fruits or sampled leaves or sampled stems

Z = Highest score scale

X = Number of infected fruits

2.2. Disease description and prevalence

Disease prevalence was studied both in the wet and dry seasons. Factors contributing to diseases of the fruit, leaf and stem were determined and described. The numbers of fruiting pawpaw trees were counted and diseased plants were defined as plants with obvious visual spoilage symptoms. Disease incidence and severity were determined. The incidence and severity data were the mean value of leaf and fruit infection on yearly assessment with data expressed in percentage over the study periods. The formula used in calculating the disease incidence and severity is:

$$\%\ \text{Leaf / fruit infection} = \frac{\text{Number of Leaves/Fruits infected} \times 100}{\text{Total no of leaves/Fruits counted}}$$

$$\text{PDI for severity} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total number of leaves/fruits} \times \text{Maximum rating value}}$$

PDI means Percent of Disease Index

2.3. Statistical Analysis

The data obtained were analysed using the Duncan's multiple range tests with $P \leq 0.05, 0.01, 0.001$ for significance.

2.4. Sample collection

Fresh fruits showing deterioration and rotting were collected at different time from the Covenant University Research and Demonstration Farm during peak disease season which also coincided with the peak harvest season of fruit (November to February). As the farm is about 500m from the Microbiology laboratory it was possible to process the samples within 6 hour of collection. Leaf samples were collected by clipping portion of inflected leaf and transported in Ziploc bags to the laboratory for further processing. Stem specimens were collected by scrapping multiple portions of disease areas. Stem scrapings were collected in sterile disposable Universal bottles.

2.5. Microscopic examination

Direct microscopic examinations of diseased tissues were done especially for fungal agents. The lactophenol cotton blue staining technique was used and prepared slides were examined at X 400 magnification.

2.6. Growth Media

Media used for bacterial cultivation include Kings agar, MacConkey agar, Nutrient agar and MRS agar. Media used for fungi cultivation were malt extract agar, potato dextrose agar (PDA) and PDA with chloramphenicol. Media used were products of Biolab and were prepared according to manufacturer's instruction.

2.7. Culture technique

Bacterial cultures were set up only for fruits while culture for fungi was done for all three samples (fruit, leaf and stem). For bacterial culture, one gram of pool spoilt specimen was homogenized in 0.89% NaCl solution and diluted serially and each dilution plated out. Incubation was aerobic at 35^{o} C for 18-48 h. Samples for fungal inoculation were prepared differently. In the first instance where growth was visible, portion of the growth and the underlining tissue were remove with a sterilised blade onto the surface of the agar plate. Alternatively, samples were homogenized in 0.89% NaCl containing 100000 units of penicillin and 0.2g/L streptomycin, and vortex at 1,500 rpm. The supernatant was decanted and the deposit re-suspended in sterile 0.89 % NaCl washed and inoculated onto fungal media. Incubation was at 27^{o} C for 2-5 days. Occasionally, plates were left for onward of 7 days. Plates for both bacterial and fungal culture were observed after incubation and colonial characteristics used for presumptive identification. Microscopic and biochemical tests were carried out where appropriate for the identification of the isolates.

Table 1. Most frequently isolated bacteria from spoilt *C. papaya* fruits

Bacteria	Frequency (%)	Predisposing conditions
Erwinia carotivora	33	Vegetation and vectors (pre-harvest)
Klebsiella spp	18	Storage conditions (post-harvest)
Enterobacter spp	8	Vectors and physical injury (pre-harvest)
Pseudomonas spp	12	Vector and physical injury (pre-harvest)
Bacillus spp	14	Vegetation (pre-harvest)
Streptococcus spp	5	-
Gram positive rods	10	-

-; not established

3. RESULTS AND DISCUSSION

Gram positive bacilli and seven genera of bacteria were isolated from *C. papaya* fruits (Table 1). Predominant was *Erwinia* followed by *Klebsiella* and *Bacillus* species. Fruit spoilage due to *Erwinia* identified post-harvest were also isolated from fruits pre-harvest and also from vectors particularly slugs. Vectors were more frequently associated with pre-harvest spoilage of fruits as the bacteria found in the spoilt fruits were also recovered from the surface and visceral of the vectors (Data not shown). *Klebsiella* was isolated from fruits mostly post-harvest. Bacteria are not frequently associated with *C. papaya* diseases especially pre-harvest and this may be attributed to the antibacterial properties in the milk latex of unripe fruits and young plants (Giordani and Siepai, 1991; Emeruwa, 1982). The bacteria isolated in this study were from plants already debilitated with prior diseases as evidence in 2013-2014 seasons. As suggested by Persley (2003), the best control measure when orchards are heavily infected is to remove the plant, fallow the field and reclaim it for subsequent cultivation. During the period of fallow cover crops may be planted that do not share same microbial pathogens with *C. papaya*.

Table 2. Disease incidences 2011-2012 and 2013-2014

Plant Part	Pathology	Disease Incidence (%) 2011-2012	Disease Incidence (%) 2013-2014
Fruit	Anthracnose Watery soft rot Black rot Stem end rot Powdery mildew	3.88 2.09 3.18 1.68 4.15	7.96 7.96 8.90 5.84 7.16
Leaf	Anthracnose Soft rot Black rot Stem end rot Powdery mildew	3.53 2.09 3.61 3.23 6.69	10.76 7.96 13.09 10.82 7.96
Stem	Stem node rot Stem canker Black rot Powdery mildew	3.79 2.07 4.13 7.69	11.02 6.95 14.13 9.42

Though powdery mildew was common to all three parts of the plant, fruit and leaf diseases were identical. The disease incidences from the two seasons studied showed higher rate in leaf and stem above incidences in fruit and therefore indicated that fruit infection may originate from these vegetative parts. Similarly, the higher disease incidences seen in 2013-2014 harvest season over 2011-2012 identifies age as critical to the vigor and resistance of *C. papaya* plant to diseases (Table 2). Figures 1 and 2 depict the state of the farm in 2011-2012 and 2013-2014; healthy plants with good fruit yield in the earlier season and infected farm two years later with poor fruit yield. Stems show symptoms of powdery mildew. Figure 3 shows leaf covered with powdery mildew and mollusc on leaf veins while Figure 4 is anthracnose on fruit post-harvest and with secondary *Rhizopus* growth on depressed water soaked regions. Anthracnose is an example of a latent fruit infection acquired in the field and symptom manifesting during storage. Vectors play major roles in the transmission of many papaya infections in the field (Picaço et al. 2003; Culik et al. 2004), the present study identified the African giant snail, slugs, beetle and millipedes as vectors. Weed control and the appropriate use of pesticides may reduce the population of these vectors in the farm.



Figure 1. Farm in 2011 with healthy stem, leaf, fruits and sparse vegetation

Table 3 which describes the severity of disease in the farm within the study period co-related well with the incidences observed in Table 2. Importantly, disease incidence is directly proportional to disease severity. However, disease severity with increasing age paints a more vivid picture of the state of the farm. While disease incidence did not result in significant difference from one harvest season to another ($p > 0.05$), it became clear with consideration of disease severity ($p < 0.001$). The economic impact of heavy infestation and infection of C. papaya orchards in Côte d'Ivoire and Hawai'i are well documented (Cunningham and Nelson, 2012; Koffi et al. 2010).



Figure 2. Farm in 2014 showing tall infected stem and few leaves and fruits on tree

Table 4 is a description of the predominant *C. papaya* diseases in the farm and the fungal agent isolated from the disease parts. *Colletotrichum gloeosporioides* was isolated mostly from anthracnose, *Phytophthora palmivora* from stem canker, *Oidium caricae* from powdery mildew while fusarium rot was caused by *Fusarium solani* and *F. oxysporium*. Watery soft rot and black rot were caused by *Rhizopus stolonifer* and *Aspergillus niger* respectively. The characteristic features of the fungal isolates are as described in Table 5. The role of these fungi in C. papaya diseases are well documented (Bautista-Baños et al. 2013; Singh et al. 2012; Teixeira da Silva et al. 2007).

Table 3. Disease severity for the period 2011-2012 and 2013-2014

Plant Part	Pathology	Disease Severity (%) 2011-2012	Disease Severity (%) 2013-2014
Fruit	Anthracnose Watery soft rot Black rot Stem end rot Powdery mildew	5.25 7.45 15.40 12.25 10.15	25.26 30.02 20.15 25.46 40.05
Leaf	Anthracnose Soft rot Black rot Stem end rot Powdery mildew	4.50 5.19 11.15 5.87 15.29	12.00 13.25 18.52 7.96 40.85
Stem	Stem node rot Stem canker Black rot Powdery mildew	15.00 5.37 5.73 4.80	35.28 12.45 15.65 45.60



Figure 4. Multiple post-harvest disease. Anthracnose was the early manifestation followed by *Rhizopus* soft rot showing white aerial mycelia visible on fruit

4. CONCLUSION

This study which is an overview of disease occurrence in our pawpaw research farm has unveiled the nightmarish experience of farmers on how their entire labour could be eroded by disease incidence. In our case, the research farm is now left fallow and reclamation processes is ongoing. We shall re-cultivate the field a year or two after when we have certified the field free of the pathogens. To recommend this practice, especially where there is pressure on land use may not yield the expected response. Consequently, alternative in this case should be considered.

Table 4. Disease description and aetiology

Description	Aetiology	Classification
Anthracnose: Brown superficial circular discolouration forming sunken water soaked lesion. Salmon pink spores form concentric pattern around lesion giving the typical bull's eye appearance	*Colletorichum gloeosporiodes*	Pre- and post-harvest
Stem rot (Canker): Occurs in young plants as damping off. Spots on stem begin as water soaked lesions, enlarge and become crusted. Stem become fibrous covered with whitish mass of fungal growth.	*Phytophthora palmivora*	Pre-and post-harvest
Powdery mildew: White superficial growth on leaf, fruit and patches of white on stem.	*Oidium caricae*	Pre-harvest
Fusarium rots: Attack both fruit and stem. Infection is systemic as mycelia mass can be seen growing within fruit cavity. Surface lesion is characterised by white mass of conidia	*Fusarium solani* *F. oxysporium*	Pre-harvest
Watery soft rot: Attacks fruit only. Water soaked lesions with irregular margin spread. At later stage lesion turns brown and fluid exudes from fruit with foul odour.	*Rhizopus stolonifer*	Post-harvest
Black rot: Brownish –black colouration on fruit. Fruit remains hard, rough, dry and with cracks.	*Aspergillus niger*	Post-harvest



Figure 3. Powdery mildew on leaf of *C. papaya* tree with diseased stem. African giant snail on leaf midrib

Table 5. Phenotypic characterization of *C. papaya* fungal isolates

Fungal isolates	Growth medium	Colonial description	Lactophenol cotton blue stained features
C. gloeosporioides	Malt extract agar	Buff aerial mycelia. Dark-brown setae. Matured spores form in 4 days incubation Buff aerial mycelia. Dark-brown setae. Matured spores form in 4 days incubation	Aerial hyaline branched septate mycelia
P. palmivora	Potato dextrose agar	Loose rosette spreading aerial hyphae	Ovoid to ovipyriform, papillate sporangiospores
O. caricae	Potato dextrose agar	White to buff mould	Septate hyaline mycelia and conidia
F. solani	Fusarium selective medium	Mycelium grey-white with sparse floccose	Oval microconidia on branched conidiophores. Conidia are sickle shaped, septate and pointed.
A. niger	Potato dextrose agar	Matured culture produce dark brown mycelial mass	Septate hyphae borne on metulae. Typical conidial heads are black and radiate

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