

## Fungal diseases of seedlings in a selected forest nursery in Port Harcourt, Nigeria

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### ABSTRACT

The incidence, causal agent, signs and symptoms of fungal diseases of seedlings in a forest nursery were assessed in the seedling nursery, Faculty of Agriculture, University of Port Harcourt between 2007 and 2011. Ten species of fungi were isolated as causal agents of stem and foliage diseases of seedlings. The ten causal agents were identified and confirmed. The fungal diseases include: damping off, leaf spot, anthracnose, powdery mildew, downy mildew, sooty mould and seedling blight. Damping off incidence was highest in five species: *Pinus caribaea*, *P. oocarpa*, *Casuarina equisetifolia*, *Calithris intratropica*, and *Nauclea diderrichii* with 67%, 56%, 58% and 76% respectively. *Napoleonaea imperialis* and *Ricinodendron hedelotii* were least susceptible to damping-off with 5% and 6% of incidence respectively. The primary means of infection is influenced by the structure of the fungal pathogen such as *conidia* and *sporangia*. The pathogens infected the stem and leaves of their respective host plants. The stomata, lenticels and epidermal cells served as the natural entry points for the pathogens. Infected seedlings were malformed, stunted, chlorotic and eventually died. Each disease inciting fungi has its characteristic symptoms which aids in identification. Since many fungi pathogens are destructive to forest seedlings in the nursery, it could be recommended that any diseases management measure, should involve silvicultural control, plant quarantine, breeding for disease resistance and the use of chemicals.

**Key words:** fungal pathogens, diseases, seedlings, forest nursery

### INTRODUCTION

Fungi play important roles in the infection and deterioration of seeds and seedlings in the nursery (Omokhua *et al*, 2011). They constitute the causative agents of different diseases in crops, fruits and other economic plants (Vashishta and Sinha, 2000). Any forest nursery is a host to

different pathogens that can cause various plant diseases. These disease-inciting organisms differ in characteristics such as cell wall, thallus, habitat, nutrition, nuclear status, distribution and reproduction. Fungal pathogens include hundreds of species which are economically important in forestry and could be classed as

*Phycomycetes*, *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* (Blanchard and Tattar, 1981). The activities of pathogens in plants are mostly chemical in nature and therefore, the disease caused by fungal pathogens in plants are the result of biochemical reactions that take place between substances secreted by the causal agent and those present within the plants (Pandey, 2009). Disease organisms can enter their hosts' plant from the soil, water and air. Poorly nourished and injured plants are very susceptible to infection in the nursery.

Several problems are associated with any study of fungal diseases on field and nursery plants in Nigeria. First and foremost, published information on seedling diseases of plants is inadequate. In addition the process is cumbersome involving macroscopic study in the field and microscopic analysis in the laboratory. Furthermore, localized outbreaks in private and government nurseries are hardly reported or published. Hence, diagnosing the right disease inciting organisms is a big problem, since their symptoms are often closely related. There is need for collaboration, systematic observations and reporting on plant diseases by scientists from different parts of the world. This creates the need for the study.

This study tends to provide some basic information on the symptomatology and aetiology of fungal diseases in a forest nursery in Port Harcourt. The objectives of this investigation are to: identify the causal agents, symptoms, signs and incidence of common fungal diseases affecting seedlings in Port Harcourt.

## MATERIALS AND METHODS

### Study Area

The study was conducted in the nursery of the Forestry and Wildlife Management

Department, Faculty of Agriculture, University of Port Harcourt. The study lasted for a period of 5 years from 2007 to 2011. The study area is located on 4° 53' 38.3" N; 6° 54' 38" E. The area is surrounded by lowland rain forest and freshwater swamp forest typical of the tropical zone. Rainfall distribution is bimodal with peak periods in July and September. Annual precipitation is very heavy and between 2000-2500 mm. Mean minimum and maximum annual temperatures are 20° C and 30° C respectively. Mean relative humidity is about 90% per annum. Soil pH vary from 4.5-5.5.

### Data Collection and Analysis

Six hundred seedlings each of timber, fruit, ornamental and medicinal plants were propagated and raised in polyethylene pots in the nursery during the study period (Table 1). Seedlings were raised under high humidity propagations before transplanting into polyethylene pots (30 x 25 x 25 mm) in the green house. The seedlings were arranged using a completely randomized design with three replicates.

Systematic observations were made and recorded on infected seedlings for symptoms of fungal pathogens. The observations per species were made from the sowing stage to early seedling growth over a period of 6 months. Two methods of investigation involving both macroscopic and microscopic procedures were employed. The macroscopic study was used to determine the signs and symptoms of the fungal pathogens on their respective host species. A hand lens (A x 15 lens) was used to observe and identify the signs and symptom of the fungi in all the seedlings infected. Observation of the characteristic structures of various fungi such as the presence of mycelia, hyphae,

conidia, conidiophore, sporangia, sporangiophore, sclerotia and uredospore was conducted.

The microscopic study of diseased seedling tissues was done under different magnifications involving careful analysis in the laboratory, to ensure correct identification of the fungal pathogens. Infected plant tissues per species from the nursery were washed and cut into 5mm segments. The segments were surface sterilized with 0.5% sodium hypochlorite solution and rinsed in three changes of sterile water. The segments were separately dried in between sheets of sterile filter paper and plated (3 pieces/plate) on fresh potato dextrose agar (PDA) medium impregnated with streptomycin and incubated at 28° C for 7 days during which the fungi growing out of the sections were isolated. The isolates were purified; pathogenicity tests and knock's postulate were carried out. All systematic observations of disease symptoms, signs and fungi isolates were verified and confirmed with existing literature no disease identification (Vashishta and Sinha, 2010; Pandey, 2009; Hill and Waller, 1994; Mehrotra and Aggarwal, 2003).

Data on damping off were statistically analysed using Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

$$\% \text{ incidence of damping off} = \frac{\text{Number of seedlings infected}}{\text{Total number of seeds sown}} \times \frac{100}{1}$$

## RESULTS

Damping off incidence was highest in five species (Table 1). These were; *Pinus caribaea*, *P. oocarpa*, *Casuarina equisetifolia*, *Calithris intratropica*, and *Nauclea diderrichii* with 67, 68, 56, 58

and 76% respectively. *Napoleonaea imperialis* and *Ricinodendron hedelotii* were least susceptible to damping-off with 5% and 6% of incidence respectively.

Ten disease inciting fungal pathogens were identified and confirmed in this study as shown in Table 2. The diseases they caused include; damping off, leaf spot, anthracnose, powdery mildew, downy mildew, sooty mould, and seedling blight. Damping off is caused by several causative agents in Port Harcourt. The fungi pathogens isolated from plant tissues are *Fusarium oxysporum*, *Pythium debaryanum* and *Phytophthora infestans*. The primary means of infection for *F. oxysporum* is the conidia, while that of *P. infestans* and *P. debaryanum* are the sporangia. The mycelium of *F. oxysporum* is extensive and branched with septate hyphae. Advanced stages of the mycelium are black with both macro and micro conidia. The mycelium of *P. infestans* is profusely branched and aseptate with thick hyphae. On the other hand, the mycelium of *P. debaryanum* represents a white fluffy appearance and coenocytic with long slender hyphae. Damping off inciting organisms were pathogenic on all plant species listed in Table 1.

Leaf spot inciting organisms invaded the leaf blade of all plant species that they infected (Table 2). The disease appeared on host plants when the seedlings were four to eight weeks old in the nursery. Two types of circular, brown and moist spots were observed. These are small and large, leaf spots which varied from 1-10 mm in diameter. Both spots were covered with fungal growth, which later enlarge to form blotches. Infected leaves turned yellow, died but persisted on mother plants for several months. The causative agents of the disease are *Cercospora parasitica* and *C. arachidicola*. The two species differ from each in size.

**Table 1. Incidence (%) of damping off of seedlings in a selected forest nursery in Port Harcourt**

<b>Plant Species</b>	<b>Incidence (%)</b>
<i>Azalia Africana</i>	49c
<i>Albizia lebeck</i>	54c
<i>Azadirachta indica</i>	33d
<i>Anona muricata</i>	38d
<i>Aframomum melegueta</i>	42d
<i>Anacardium occidentale</i>	33d
<i>Casuarina equisetifolia</i>	56c
<i>Carica papaya</i>	34d
<i>Calithris intratropica</i>	58c
<i>Cola pachy-carpa</i>	18f
<i>Chrysophyllum albidum</i>	24e
<i>C. delevoyi</i>	26e
<i>Dacryodes edulis</i>	15f
<i>Daniella ogea</i>	32d
<i>Detarium macro carpum</i>	38d
<i>Entandrophragma angolense</i>	19f
<i>Gmelina arborea</i>	56 c
<i>Garcinia kola</i>	53c
<i>Hura crepitans</i>	52c
<i>Irvingia gabonensis</i>	50c
<i>I. wombolu</i>	54c
<i>Kigelia Africana</i>	51c
<i>Mansonia altissima</i>	18f
<i>Mangifera indica</i>	16f
<i>Moringa oleifera</i>	24e
<i>Napoleonaea imperialis</i>	5g
<i>Nauclea diderrichii</i>	76a
<i>Pinus caribaea</i>	67b
<i>P. oocarpa</i>	68b
<i>Psidium guajava</i>	36d
<i>Persea Americana</i>	22e
<i>Riciodendron heudelotii</i>	6g
<i>Tamarindus indica</i>	16f
<i>Tectona grandis</i>	48c
<i>Treculia Africana</i>	37d
<i>Terminalia catappa</i>	20e
<i>T. mantaly</i>	21e
<i>T. ivorensis</i>	19f

Means with same letter do not differ significantly at 5% level of probability

Table 2: Fungal diseases of seedlings in University of Port Harcourt nursery, Port Harcourt

NAME OF DISEASES	CAUSAL	CLASS/FAMILY	SYMPTOMS	HOST SEEDLINGS
Damping Off	<i>Fusarium oxysporum</i> <i>Pythium debaryanum</i> <i>Phytophthora infestans</i>	<i>Deuteromycetes Tuberculariaceae</i> <i>Oomycetes Pythiaceae</i> <i>Oomycetes Pythiaceae</i>	Stem bases close to soil surfaced pinched and shriveled. Seedlings collapse, die and decay	All plants species listed in Table 1
Leaf spot	<i>Cercospora personata</i> <i>C. arachidicola</i>	<i>Deuteromycetes Dematiaceae</i> <i>Deuteromycetes Dematiaceae</i>	Necrotic lesions appear both on the upper and lower leaf surfaces. Older spots have yellow halo around them.	<i>Tectona grandis</i> <i>Gmelina arborea</i> <i>Khaya species</i> <i>Terminalia species</i> <i>Azela africana</i> <i>Daniellia oliveri</i>
Anthraxnose	<i>Collectotrichum gloeosporioides</i>	<i>Deuteromycetes Melanconiaceae</i>	Black or brown sunken angular, necrotic lesions appear on leaf surfaces in advance stages. spots elongated, veins darkened and leaves fall out.	<i>Garcinia kola</i> <i>Cola pachycarpa</i> <i>Kigelia africana</i> <i>Delonix regia</i> <i>Leucaena leucocephala</i> <i>Khaya grandifoliola</i> <i>G. arborea</i>
Powdery mildew	<i>Erysiphe polygoni</i>	<i>Ascomycetes Erysiphaceae</i>	White powdery growth on older leaf surfaces. Chlorosis and stunting of seedling	<i>Terminalia ivorensis</i> <i>T. grandis</i> <i>G. arborea</i> <i>K. africana</i>
Downy mildew	<i>Peronospora parastica</i>	<i>Oomycetes Peronosporaceae</i>	Grayish or purple brown downy spots on the lower surface of the lower older leaves. Yellow lesions appear on upper part of leaves	<i>Entandrophragma angolense</i> <i>Khaya grandifoliola</i> <i>R. heudelotii</i> <i>K. africana</i> <i>Nauclea diderrichii</i> <i>Detarium macrocarpum</i> <i>Treculia africana</i>
Sooty mold	<i>Aspergillus niger</i>	<i>Deuteromycetes Aspergillaceae (Eurotiaceae)</i>	Older leaves develop black to dark brown spots with concentric rings, infection spread with sooty mold appearance on leaves.	<i>Chrysophyllum species</i> <i>Irvingia gabonensis</i> <i>Dacryodes edulis</i> <i>Treculia africana</i> <i>Persea americana</i>
Seedling blight	<i>Alternaria solani</i>	<i>Deuteromycetes Dematiaceae</i>	circular necrotic and brown lesions with concentric zonation appear on leaves and stem, followed by shedding of leaves	<i>Mansonia altissima</i> <i>Khaya grandifoliola</i> <i>Albizia lebbek</i> <i>Leucaena leucocephala</i> <i>Dennettia tripetala</i>

shape and colour of necrotic lesions as well as conidia formation and nature of the mycelium. Very wet conditions favoured the severity of infection. The mycelium could be internal and external with black globular mass of hyphae.

Anthraxnose is another serious fungal seedling disease in the nursery in Port Harcourt. Sunken black spots appeared on the foliage of infected seedlings. The disease is associated with moist and very warm conditions. The causative agents are *Colletotrichum* species (Table 2). The fungi attack both fruit and timber forest species in the nursery. The mycelium is internal, branched and septate. The conidia are the primary means of infection.

Powdery mildew is a common foliage disease of seedlings affecting a wide range of plants in the nursery (Table 2). The causative agents are external parasites in which the mycelium forms whitish patches on the upper surface of leaves of seedlings. In this study, the upper surfaces of old leaves in infected seedlings were covered with powdery white growth which spread rapidly to other parts of the plant. Affected parts became chlorotic and stunted in growth. In advanced stages, the seedling became dirty and consequently shed their leaves. The causative agent is *Erysiphe polgoni*. The disease was favoured by warm and dry weather conditions in Port Harcourt. The severity was mainly between the drier months of November and March of every year. Downy Mildew is caused by internal obligate parasites. They are characterized by superficial down growth of conidia in host lesions. The causative agent is *Peronospora parasitica*. The means of primary infection is conidia (Table 2).

The causative agent of seedling sooty mould in the nursery is *Aspergillus niger* (*Euotium* species). The primary

means of infection by the black fungus are the conidia. The symptoms appeared on older leaves with black or dark brown spots. The crown later collapsed and rot with characteristic sooty appearance. High humidity favoured its development in Port Harcourt. The mycelium is extensively branched, bright coloured with interwoven mass of hyphae.

Seedling blight at this nursery is caused by *Alternaria solani*. Lesions occurred on leaves and stems which are circular, necrotic and brown with concentric zonation. Leaves were later shed. Mycelium is branched and short with septate hyphae. The pathogen is soil-borne and favoured by wet periods. The visible signs on seedlings are large conidia.

## DISCUSSION

Forest disease inciting organisms are diverse and common in the soil media used for propagation in Port Harcourt (Table 1). Damping off is a very serious disease caused by some pathogens. In this study, all tree species propagated in Port Harcourt are susceptible to damping off disease (Table 1). The disease is caused by *Fusarium*, *Pythium* and *Phytophthora* species. Seedlings in the nursery can suffer from various fungal diseases during propagation, no matter the part of plants attacked; the impact on the seedlings could be disastrous. Infected seedlings became malformed, chlorotic, deteriorated, stunted, and eventually died.

Diverse groups of fungi are responsible for various seedling diseases in Port Harcourt. They consist of non-green nucleated thallophytes whose mode of feeding on seedlings is either saprophytic or parasitic. The saprophytes (saprobies) live and grow on dead organic matter. The parasites on the other hand, live in their host or grow on them or both. The symptoms of a disease are the visible

effects which the fungi induce on the young seedlings. Nursery disease symptoms could be localized (individual lesions are restricted to certain plant parts) or systemic (lesions affect all or most of the seedlings).

The fungal diseases observed in this study infected the stems and leaves of seedlings in the nursery. Many scientists have reported on the causes and impacts of fungal diseases on seedlings from different parts of the world (Omokhua, *et al* 2011; Canadian Council of Forest Ministers, 2000; Woods *et al.*, 2000; Gordon *et al*; FAO 2006, 2007; Tainter, 2003; Ostry and Juzwik, 2008; Callan, 2001; Mohan, 2008; Schmidt, 2003; Mehrotra and Aggarwal, 2006; Gilbert, 1995). One of the main objectives of forest disease management is to reduce or prevent disease incidence to the barest minimum. Therefore, any effective disease management option should begin with the use of practices that prevent specific seedling diseases rather than those that are geared towards curing them. One outstanding advantage of disease control in forest plants in the nursery is that the diseases are treatable unlike in the field where they are difficult to treat due to inaccessibility of the stands, vast extension of forest and high cost of labour which can render any control method ineffective or relatively impracticable.

As the seedlings grow into maturity, the parasites develop in the tissues of their host plants. The symptoms gradually appear and manifest with age of infections, the microbial characteristic of the parasites vary from one species to another. The natural openings in plants which serve as entry points for the pathogens include the stomata, lenticels and epidermal cells.

A forest nursery is an important establishment for production of seedlings to support afforestation programme

anywhere in the world. Its protection from nursery diseases is an essential aspect of Silviculture. Therefore, seedling diseases in the nursery should be properly identified so that appropriate control strategies can be adopted to reduce their impact.

In conclusion, damping off was the most destructive disease of seedlings in the nursery under investigation because it caused more damage and destruction to seedlings than other fungal diseases. In the nursery, fungal pathogens can infect several nursery seedlings through seeds, soil, vegetative materials, and mechanical means such as contact of infected and healthy plant as well as through vectors such as aphids, mealybugs and other insects. The following disease management measures are recommended.

- a) destruction and proper disposal of diseased seedlings,
- b) avoidance of the use of untreated domestic compost in potting mixture,
- c) constant weeding,
- d) avoidance of over watering,
- e) avoidance of damage to stems and roots during pricking and transplanting of germinants,
- f) thinning of excessive germinants,
- g) nursery beds should not be sited in waterlogged areas,
- h) avoidance of overcrowding of nursery stock,
- i) sterilization of all growth media,
- j) there should be legal restriction of the movement of seeds and seedlings from one area to another through adequate inspection by relevant government agency
- k) selection and breeding of plant species that show resistance to specific diseases should be done

- l) nursery disease could be controlled by chemical measures such as the application of fungicides and fumigants on seeds and seedlings. The use of Dithanet M45 has proved very effective against damping off in Port Harcourt.

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