



Research Article

Seedborne Fungi, *Cercospora* Leaf Spot of Seedlings and Effect of Substrates on Germination, Seed Infection and Growth of *Lophira alata* (Azobé) in Cameroon

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ABSTRACT

Seedborne fungi are among the main causes of low productivity of forestry nursery in the world. This study was carried out with the objective to contribute to improving the productivity of *Lophira alata* in Cameroon through the knowledge of seedborne fungi and diseases affecting the germination and growth of Azobe's seedlings in different substrates. To achieve this, the following substrates: red forest soil (RFS), cultivated soil (CS), a mixture of red forest soil and sand (RFSS) in a ratio of 1/1 and a mixture of red forest soil, sand and poultry manure in a ratio of 3/1/1 (RFSSP) were used to evaluate the germination and the infection of seed bank of the national school of forestry (Mbalmayo, Cameroon), growth and *Cercospora* leaf spot incidence and severity of seedlings in the nursery. Results showed that frequently isolated fungi were *Fusarium oxysporum* (40-75%), *Rhizoctonia solani* (10-35%) and *Aspergillus niger* (10-25%) on the seeds and *Cercospora* sp (86%) on seedlings. Germination and growth rate of Azobe is very low while seed infection is very high. The substrate RFS gave significantly ($P < 0.05$) higher germination (18.6%) with the lower seed infection (81.4%), 62 days after sowing (DAS) than the other substrates. There was no significant difference in collar diameter and number of leaves both at 70 and 84 DAS between the substrates. The height of seedlings was significantly higher in RFSSP (14.8 and 15.7 cm at 70 and 84 DAS respectively) compared to other substrates; however, with this substrate, *Cercospora* leaf spot incidence (99.6%) and severity (68.1%) were higher at 84 DAS. The results provide basic information needed for the development of control measures of seedborne fungi and *Cercospora* leaf spot of *L. alata* in Cameroon.

Key words: Azobe, *Cercospora* leaf spot, Germination, Seed borne fungi, Substrates

INTRODUCTION

Lophira alata Banks ex P. Gaertn. (commonly known as azobé, ekki or red iron wood) is a species of plant in the *Ochnaceae* family found in subtropical or tropical moist lowland forests. The species is monoecious, the flowers are insect-pollinated and regenerate easily on exposed areas (Anon, 1954). Azobé is an important species of interest because of its diverse uses, occupying the 4th place on the list of exploited wood in Cameroon with 5.1% the volume of harvested wood, representing about 17500 m³. The wood is extremely hard and used for the construction of railways and bridges. Due to its over exploitation, Azobé (*Lophira alata*) is one of the forest species classified in the Red List of International Union for Conservation of Nature (IUCN), the reason why its

protection and conservation is recommended. Although *L. alata* needs full sunlight to grow, seedlings can persist for some time in the shady undergrowth and resume growth if breaks in the canopy occur. Azobé prefers deep hydromorphic soils and regenerates on well drained humid soils (Dupuy, 1998). The species prefers sandy and sandy-clay soils and also poor and acidic soils and constituted a good indicator of poor soils (Kammegne, 2004). The germination rate varies between 85% and 95% and can reduce to 25% after 4 months of conservation (Mensbruge, 1966; Doumenge and Séné, 2012). A field survey carried in 2010 at Lomie (East Cameroon) shows that seeds and seedlings of the species are too susceptible to disease in natural stands than in forestry nurseries (Djeugap, 2010). In forest sciences, seeds and seedling diseases are very damageable for forest regeneration and

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productivity of forest species. Many fungi are carried through seeds into forest nurseries and become established on seedlings and later spray to plantations and forests causing heavy damage. It is therefore necessary to collect disease-free mature seeds to constitute a gene bank and store them appropriately for further uses. Gene banks are facilities for long-term storage of seeds and seeds must be held under conditioned storage, and tested for viability every 5 to 10 years (Doidjode, 2001; Schmidt, 2007). Apart from these seedborne fungal pathogens, soil-borne fungal pathogens have also been shown to be devastating by attacking young seedlings in the forest due to their tender tissues and as they often have difficulty in establishing themselves. Unfortunately, no research has been carried out in the study of seeds and seedling diseases of this timber species. However, very few studies have been carried out on seed and seedling diseases of some forest species in Cameroon and beyond. In fact, previous studies reported seed rot and shoot blight of seedlings of *Ricinodendron heudelotii* (Djeugap *et al.*, 2014; Djeugap *et al.*, 2016), seedborne fungi of *Pericopsis elata* (Djeugap *et al.*, 2017a), *Cercospora* leaf spot on seedlings of *Eucalyptus saligna* and *Prunus africana* (Djeugap *et al.*, 2017c). In Ghana, many pathogenic fungi (*Rhizoctonia solani*, *Cercospora* sp, *Aspergillus niger*, *A. flavus*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Verticillium* sp. and *Fusarium* spp.) were reported on seeds and seedlings of *Albizia zygia*, *Cedrella odorata*, *Chlorophora excelsa*, *Entandrophragma utigle*, *Gmelina arborea*, *Khaya grandifoliola*, *Mansonia altissima*, *Pericopsis elata*, *Terminalia ivorensis* and *Triplochiton scleroxylon* (Gyimah, 1987). Leaf blight on *Eucalyptus* spp. caused by a *Calonectria* species was also reported in Ghana (Apetorgbor and Roux, 2015). In Nigeria, leaf spot and dieback caused by *Colletotrichum capsici*, *Fusarium solani* and *Lasiodiplodia theobromae* were reported on *Ceiba pentandra* seedlings while damping-off caused by *Fusarium oxysporum* was reported on seedlings of *Pinus caribaea* and *Pinus oocarpa* (Omokhua *et al.*, 2009). The aim of this study was to identify seedborne fungi associated with the seed bank of the national school of forestry and seedlings of *L. alata* in nursery. This will allow establishing a foundation to support programs aimed at tree improvement, plantation creation and disease control in the distribution areas of the species in Cameroon.

MATERIALS AND METHODS

Seed collection and substrates for germination and seedling growth

Seeds were harvested from the seed bank collection of the National School of forestry (NSF), Mbalmayo (Cameroon). They were collected 3 to 4 months ago in different trees bearing mature fruits at the arboretum of the NFS and stored at ambient temperature (22±2°C) in envelopes. The arboretum is located between 2.1° to 5.8° N and 10.5° to 16.2° E at 600-800 m elevation; the soil is lateritic (IRAD Mbalmayo, 2009). Four substrates for germination and seedling growth were considered: cultivated soil (CS) obtained from cultivated land; Red forest soil (RFS) obtained from the arboretum of the National School of Forestry; a mixture of red forest soil

and sand in the ratio 1:1 (RFSS) and the mixture of Red forest soil, sand and poultry manure at a ratio of 3/1/1 (RFSSP). Germination tests were carried out in the nursery of the NSF (which is near the arboretum) in a seed bed previously sprayed with Calthio C 50 WS. This insecticide-fungicide is recommended for seed treatment before sowing against insects and fungi, responsible for damping-off.

Sowing of seeds and seedlings transferred into polyethylene bags

Before sowing of seeds, they were selected to remove the infected ones. This was done by identifying physically seeds that have holes and any sign and symptoms of an infection. Then, given the fact that Azobe has winged seeds, the wings were removed and seeds were soaked in a basin of water for 5 minutes. From this test, the viable seeds should sink due to their density while the non-viable should float. One seed was sown in each hole at a depth of 1 cm to ease the outbreak of the first shoot from the ground (Doumenge and Séné, 2012). After sowing, a shade of 2 m height was constructed with sticks and palm fronts to create a suitable environment for seed germination and growth of seedlings. Two hundred seeds were considered per substrate in four replicates. One week after germination, young seedlings were removed from seed bed and transferred into polyethylene bags of 20 cm in diameter containing the different substrates (Djeugap *et al.*, 2017a). Each polyethylene bag received one healthy seedling and the bags were distributed in the nursery following a complete randomized design with four replicates.

Data collection in the nursery

Data collected were seed germination, infection rate, growth parameters, severity and incidence of *Cercospora* leaf spot on seedlings. Germinated seeds and cumulative germination percentages were counted every 3 days for 37 days; a seed was considered germinated when the radicle became visible (Mbaye *et al.*, 2002). Seed infection was recorded at the end of germination i.e. at 37 days after sowing (DAS). Disease parameters were collected on weekly basis until 56 DAS. The following formula was used:

Germination (%) = (Number of germinated seeds / Total number of seeds sowed) × 100. Seed Infection (%) = (Number of non-germinated and infected seeds / Total number of grains sowed) × 100. Disease incidence (%) = (Number of plants infected / Total number of plants examined) × 100 and disease severity (%) = (surface in percent of tissue infected over the total surface of tissues considered) (Campbell and Madden, 1990). Growth parameters collected on weekly basis during 56 DAS were: height, collar diameter and number of leaves of a plant.

Isolation and identification of fungi

Infected seeds and seedling's leaves were collected under mature trees in the arboretum of NSF, Mbalmayo, and carried to the laboratory for pathogen isolation. Infected seeds in the seed bed that do not germinate were also collected with respect to substrates. Isolation was carried out on sterilized potato dextrose agar medium (PDA) after disinfection of infected organs in sodium

hypochlorite solution (5%, 3 minutes) (Korsten *et al.*, 1994). After purification on the same medium, pure fungal isolates obtained were identified based on morphological characteristics; mycelium structure and spore morphology using keys of fungi identification (Alexopoulos *et al.*, 1996; Champion, 1997). The frequency of occurrence (FO) of each fungus was calculated using the formula, $FO = (NF/NT) \times 100$, where FO represents the frequency of occurrence (%) of a fungus, NF is the total number of samples from which a particular fungus was isolated and NT is the total number of samples from which isolations were carried out (Iqbal and Saeed, 2012).

Data analysis

Data collected (germination, seed infection, growth parameters, disease incidence and severity) were typed in Microsoft Excel and submitted to analysis of variance. Data in percent were transformed (arcsine) before analysis. The software SPSS version 17.0 was used for all analysis and mean separation was made using the Duncan multiple range test at 5%.

RESULTS

Seedborne fungi on sown and unsown seeds and associated fungi on the leaves of *L. alata*

Seven different fungi reported as pathogenic to plants were identified on sown and unsown seeds of Azobe amongst with the species *Fusarium oxysporum* and *Rhizoctonia solani* being frequently associated with infected seeds, both for sown and unsown seeds. The highest isolation frequency was 40-75% for *F. oxysporum* and 10-35% for *R. solani*. Two fungi (*Aspergillus niger* and *Cercospora* sp) were isolated on infected leaves of seedlings with *Cercospora* sp possessing the highest isolation frequency (86%) (Table 1).

Effect of substrates on germination and seed infection

Generally, the germination rate of *L. alata* seed was too low. It increased with time in all the substrates and ended at 62 days after sowing (DAS). There was no significant difference in germination in all the substrates at 35 and 44 DAS. However, at 62 DAS, germination was significantly higher ($P < 0.05$) in red forest substrate (18.6%) compared to other substrates (Table 2). Germination was lowest in RFSSP (7.3%). Seed infection was significantly lower in RFS (81.4%) compared to other substrates. Substrates RFSS (91.7%) and RFSSP (92.7%) gave the highest seed infection rate at 62 DAS (Table 2).

Effect of substrates on growth parameters of seedlings

The effect of substrates on collar diameter, number of leaves and height of Azobe seedlings is presented in table 3. Generally, it was observed that the growth of Azobe seedlings is very slow in all the four substrates. There was no significant difference ($P > 0.05$) of collar diameter and number of leaves both at 70 and 84 DAS for the all the substrates. However, there was a significant effect ($P < 0.05$) of substrates on the height of the seedlings. In fact, the substrate RFSSP gave the highest seedlings height (14.8 and 15.8 cm) both at 70 and 84 DAS respectively compared to the other substrates (Table 3).

Table 1: Seedborne fungi and fungi associated with seedling's diseases of *Lophira alata*.

Seeds/ leaves	Substrates*Fungi	Frequency of occurrence (%)
Unsown seeds	<i>Aspergillus niger</i>	20
	<i>Cercospora</i> sp	20
	<i>Fusarium oxysporum</i>	40
	<i>Phoma</i>	10
	<i>Rhizoctonia solani</i>	10
	Total	100
	<i>Cercospora</i> sp	5
	RFS <i>Fusarium oxysporum</i>	75
	<i>Phoma</i> sp	25
	Total	100
Sown seeds	<i>Aspergillus niger</i>	25
	CS <i>Gonatotobrys</i> sp	60
	<i>Rhizoctonia solani</i>	15
	Total	100
	RFSS <i>Fusarium oxysporum</i>	65
	<i>Nigrospora</i> sp	35
	Total	100
	RFSSP <i>Aspergillus niger</i>	10
	<i>Fusarium oxysporum</i>	55
	<i>Rhizoctonia solani</i>	35
Seedling's leaves	Total	100
	<i>Aspergillus niger</i>	14
	<i>Cercospora</i> sp	86
	Total	100

*CS = cultivated soil, RFS = Red forest soil, RFSS = mixture of red forest soil and sand in the ratio 1/1 and RFSSP = mixture of Red forest soil, sand and poultry manure at a ratio of 3/1/1.

Table 2: Seed germination and infection rate with respect to different substrates.

Substrates	Germination rate (%)			Infection rate (%)
	35 DAS	44 DAS	62 DAS	62DAS
RFS	5.2 ± 2.5 ^a	11.5 ± 3.5 ^a	18.6 ± 3.5 ^a	81.4 ± 2.7 ^b
CS	4.2 ± 1.6 ^a	9.4 ± 2.3 ^a	12.5 ± 3.4 ^b	87.5 ± 4.2 ^a
RFSS	6.9 ± 2.0 ^a	7.7 ± 3.9 ^a	8.3 ± 2.5 ^c	91.7 ± 4.3 ^a
RFSSP	6.3 ± 2.2 ^a	7.3 ± 1.8 ^a	7.3 ± 1.8 ^c	92.7 ± 4.9 ^a

*Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test 5 %. CS = cultivated soil, RFS = Red forest soil, RFSS = mixture of red forest soil and sand in the ratio 1/1 and RFSSP = mixture of Red forest soil, sand and poultry manure at a ratio of 3/1/1.

Disease incidence and severity on seed bed seedlings

At 56 and 84 DAS, there was a significant difference of disease incidence among substrates. For disease severity, significant differences were observed only at 84 DAS. The Substrates RFSSP showed the highest incidence and disease severity both at 56 and 84 DAS compared to the other substrates. For RFSSP substrate, disease incidence and severity were 100 and 68.1% respectively at 84 DAS. There was no significant difference of disease incidence and severity among RFS, CS and RFSS substrates both at 56 and 84 DAS (Table 4). Disease incidence and severity increased with time in all the substrates tested.

DISCUSSION

Seed germination was relatively lower (7.3-18.6%) compared to the average germination rate usually obtained in *L. alata*. In fact, germination rate in Azobe is generally between 80 and 95%. This could be due to storage

conditions of seed at the National School of forestry; they were stored at ambient temperature for more than 3 months in envelopes. Also, for oleaginous seeds, their viability drops quickly because of the rapid peroxidation of lipid content (Doumenge and Séné, 2012). Storage temperature, storage duration and seed moisture content play an important role for seed viability. Depending on plant species, the suitable storage temperature for seeds range from 4 to 10°C (Lui *et al.*, 2011; Pradhan and Badola, 2012). In regions characterised by periods of air temperature and relative humidity higher than 25°C and 65-70%, respectively, as in humid tropics, storage of more than 3-4 months may be harmful to seed viability (Abba and Lovato, 1999). Seeds stored for long-term at unsuitable temperature/condition significantly affects their germination capacity as indicated by Bradbeer (1998); nonetheless, it varies greatly by species and storage conditions (Siddique and Wright, 2003). The low germination rate and the high infection rate of the seeds obtained in this study may be due to both shelf-life and storage temperature not recommended for conservation for a long period of time. In fact, it was established that seed longevity is a function of species, initial seed quality (production and conditioning), and storage environment. They must be stored in sealed containers and in an environment where the temperature and relative humidity are controlled (Schmidt, 2007). Seeds from the Forestry Unit's collection of the National Forestry School were not stored as required. They were sealed in envelopes and left at room temperature.

Results also show that substrates significantly influence the collar diameter and height of Azobe seedlings. Seedlings height were higher in RFSSP substrate compared to others substrates and there was no significant differences of collar diameter between RFS and RFSSP substrates. In fact, RFSSP substrate is made of the mixture between red forest soils (which is rich in nitrogen content in poultry manure). Forest tree species take up nitrogen both in NH_4^+ and NO_3^- forms during their growth. It was shown that increase in nitrogen deposition has increased tree growth by 12% (Nissinen and Hari, 1998). However, substrates made of different mineral components are advisable to be used for seedlings growth since each nutrient has a specific role to play in the growth and the well-being of the plant. Generally, organic manure with livestock by-products increases soil pH and nitrogen, available phosphorus, exchangeable potassium, calcium, and magnesium which are suitable for plant growth (Eghball *et al.*, 2002). Poultry manure allows plants to use the nutrients for a long time, due to its slow decomposition, and reduces the loss of what is not utilized by the plants (Bhandari *et al.*, 2002). The advantage of substrate supply by poultry manure on seedlings growth

compared to the other substrates was also reported on *Liriodendron tulipifera* (Si *et al.*, 2016).

The study also reveal that most fungi that affected seeds and leaves originated from seed infection before sowing especially *Fusarium oxysporum* and *Cercospora sp* though species like *F. oxysporum* could originate from the soil as shown by James *et al.* (1987). *Fusarium sp*, *Aspergillus niger* and *Cercospora sp* observed on seeds of Azobe were also associated with seedborne fungi of *Monodora myristica* and *Ricinodendron heudelotii* (Djeugap *et al.*, 2017b; Zena *et al.*, 2017) and of *Pericopsis elata* (Djeugap *et al.*, 2017a). *Fusarium sp* was previously reported as the main cause of the wilt disease of *Hardwickia binata* and leaf blight disease of *Terminalia capitata* (Rai and Mamatha, 2005) and on *Pinus radiata* seedlings in the nursery (Lori *et al.*, 1999). Also, *Cercospora sp* happens to be one of the most common foliage diseases that damage forest nursery. It was reported on *Dendrocalamus strictus* by Rai and Mamatha (2005) and on teak nurseries in India (Murthy and Lokesh, 2013). *Fusarium oxysporum* was isolated in all sown and unsown seeds with the highest frequency of occurrence. In fact, this species has been associated with important diseases in forest nurseries for decades. It causes several different types of diseases, including pre- and post-emergence damping-off, cotyledon blight, stem and root decay of young seedlings, and root disease of older seedlings (James 1987; James *et al.*, 2002). Damage has especially been severe on pine species, including ponderosa (*Pinus ponderosa*) and western white (*P. monticola*). It is a cosmopolitan and polyphagous fungal species (Stewart *et al.*, 2006). *Rhizoctonia solani* was also reported as seedborne fungi on many forest seedlings where it causes damping-off (Hietala and Sen, 1996; Motta *et al.*, 2007). It could be advisable to sow seeds within the month of collection to avoid the risk of contamination. In the case of seed conservation, seed treatments can be done, such as seed drying, the use of chemical or heat treatment and stored at appropriate temperature and relative humidity to avoid or reduce infections.

Conclusion

Seedborne fungi and substrates management are essential for sustainable seedlings production in nursery. The main fungi isolated in *Lophira alata* seeds were *Fusarium oxysporum*, *Rhizoctonia solani* and *Aspergillus niger* while seedlings were mostly infected with *Cercospora sp*. The substrate "Red Forest Soil" (RFS) is suitable for seed germination than the other substrates while substrate made up of a mixture of "red forest soil, sand and poultry manure in a ratio of 3/1/1" (RFSSP) was suitable for seedlings growth. Appropriate control measures

Table 3: Effect of substrates on growth parameters of Azobe's seedlings in the seedbed.

Substrates	Collar diameter (mm)		Number of leaves		Height (cm)	
	70DAS	84DAS	70DAS	84DAS	70DAS	84DAS
RFS	1.9±0.3 ^a	1.9± 0.2 ^a	3.3±0.9 ^a	3.8±1.0 ^a	10.6±2.7 ^b	11.2± 2.2 ^b
CS	1.4±0.4 ^{ab}	1.5±0.6 ^a	3.3 ±0.9 ^a	3.4±0.9 ^a	9.8±3.3 ^b	10.0±3.1 ^b
RFSS	1.1±0.1 ^b	1.5±0.5 ^a	3.2±0.7 ^a	3.5±0.9 ^a	9.9±3.4 ^b	10.0± 3.5 ^b
RFSSP	1.4±0.3 ^a	1.8± 0.3 ^a	3.3±1.2 ^a	4.3±1.2 ^a	14.8±3.4 ^a	15.7± 3.4 ^a

*Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test at 5%. CS = cultivated soil, RFS = Red forest soil, RFSS = mixture of red forest soil and sand in the ratio 1/1 and RFSSP = mixture of Red forest soil, sand and poultry manure at a ratio of 3/1/1.

Table 4: Incidence and severity of *Cercospora* leaf spot on seedlings of *Lophira alata*, 56 and 84 days after sowing.

Substrates	Disease incidence (%)		Disease severity (%)	
	56DAS	84DAS	56DAS	84DAS
RFS	9.5±5.1 ^{b*}	63.5±8.3 ^b	14.3±5.7 ^a	48.5±9.7 ^b
CS	27.8±7.1 ^b	72.2±12.0 ^b	21.7±8.0 ^a	40.7±8.8 ^b
RFSS	46.7±9.0 ^{ab}	60.0±9.4 ^b	22.0±6.7 ^a	35.3±8.1 ^b
RFSSP	66.7±11.8 ^a	99.6±0.4 ^a	20.9±5.4 ^a	68.1±11.6 ^a

*Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test 5%. CS = cultivated soil, RFS = Red forest soil, RFSS = mixture of red forest soil and sand in the ratio 1:1 and RFSSP = mixture of Red forest soil, sand and poultry manure at a ratio of 3/1/1.

should be envisaged against seedborne fungi and *Cercospora* leaf spot of *L. alata* in order to reduce their negative impact on nursery productivity in Cameroon.

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