Insect pests and mycoflora of oilbean (*Pentaclethra macrophylla* Benth.) pods and seeds in southeastern parts of Nigeria

Ikechukwu Cyriacus OKWULEHIE

Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Abia State, Nigeria phylyke@yahoo.com

Insect pests and mycoflora of oilbean (*Pentaclethra macrophylla* Benth.) pods and seeds in southeastern parts of Nigeria.

Abstract — Introduction, Pentaclethra macrophylla, Mimosaceae, is the only species of the genus present in West Africa. The plant's diseases are not well known. The numerous uses of the plant's seeds and pods aroused our interest in investigating its pests and mycoflora, which would constitute a drawback to the plant's production, value and use. **Materials and method**. Mature pods of *P. macrophylla* were collected from different locations in Nigeria and tree-top observations were also made for studying the pests present. Isolation, characterization and identification of the mycoflora were done by plating washings from pods, skin and seeds on different media and incubating for 2 to 3 days at 28 °C. Control plating was done with sterile peptone water. Streptomycin was incorporated in the test and control media to avoid bacteria. Some pods and seeds were also stored before being treated as above. A pathogenicity test was also carried out. Results and discussion. Many insect species and pathogens were found to attack the pods and seeds of the plant. The major insect pests identified were Cossus cadaribae, Sitophilus spp., Spodoptera exempta and silkworms. The major fungal pathogens included Aspergillus niger, Rhizopus stolonifer, Cladosporium berbarum, Penicillium sp., Botryodiplodia theobromae and Fusarium sp. Some of the insect pests skeletonize the green pods, depriving them of photosynthetic surfaces; some bore into the pods, eating up and damaging the seeds' fleshy cotyledons; others lacerate the pods, causing lesions of varying sizes and shapes, thereby creating portals for the penetration of fungal and bacterial pathogens which invade and rot the predisposed seeds. **Conclusion**. Our study established many pests and mycoflora which attack P. macrophylla pods and seeds. They might have been the cause of the observed symptoms and damage, and the cause of the reduction in the crops planted, reported by previous authors.

Nigeria / Pentaclethra macrophylla / pests / microbial flora / identification

Insectes parasites et mycoflore des gousses et graines de *Pentaclethra macrophylla* Benth. dans le sud-est du Nigéria.

Résumé — Introduction. *Pentaclethra macrophylla*, Mimosaceae, est la seule espèce du genre présente en Afrique occidentale. Les maladies de cette plante ne sont pas bien connues. Les nombreuses utilisations des gousses et des graines de l'arbre nous ont conduit à étudier les parasites et la mycoflore de ces productions, qui constitueraient un obstacle à la production de l'espèce, à sa valorisation et à son utilisation. **Matériel et méthodes**. Des gousses mûres de *Pentaclethra* macrophylla ont été collectées en différents lieux du Nigéria et des observations de la partie supérieure de l'arbre ont été également faites pour étudier les parasites présents. L'isolement, la caractérisation et l'identification de la mycoflore ont été faits par des ensemencements d'eau de rincage des gousses, de la peau et des graines sur différents milieux incubés pendant 2 à 3 jours à 28 °C. L'ensemencement témoin a été fait avec de l'eau peptonée stérile. De la streptomycine a été incorporée dans les milieux d'essai et témoin pour éviter le développement de bactéries. Conjointement, quelques gousses et graines ont été stockées en laboratoire avant d'être traitées comme précé-demment. Un essai de pathogénicité a été également effectué. **Résultats et discussion**. Beaucoup d'espèces d'insectes et de microbes pathogènes ont été trouvés comme attaquant les gousses et les graines des plants. Les principaux insectes parasites identifiés ont été Cossus cadaribae, Sitophilus spp., Spodoptera exempta et des vers à soie. Les principaux pathogènes fongiques ont été Aspergillus niger, Rhizopus stolonifer, Cladosporium herbarum, Penicillium sp., Botryodiplodia theobromae et Fusarium sp. Certains des d'insectes parasites squelettisent les gousses vertes les privant de leurs surfaces photosynthétiques ; certains percent les gousses en se nourrissant et endommagent les cotylédons charnus des graines ; d'autres lacèrent les gousses causant des lésions de tailles et de formes variables qui sont autant de portails pour la pénétration des pathogènes fongiques et des bactéries qui envahissent les gousses et décomposent les graines accessibles. **Conclusions**. Notre étude a montré que beaucoup de parasites et d'éléments de la mycoflore attaquaient les gousses et les graines de Pentaclethra macrophylla. Ils pourraient être la cause des symptômes et des dommages observés, et expliquer la régression de la plante cultivée, rapportée par des travaux antérieurs.

10.1051/fruits:2004003 Nigéria / Pentacleti

Nigéria / *Pentacletbra macrophylla* / organisme nuisible / flore microbienne / identification

Fruits, 2004, vol. 59, p. 25–30 © 2004 Cirad/EDP Sciences All rights reserved DOI: 10.1051/fruits:2004003

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1. Introduction

Pentaclethra macrophylla, of the family Mimosaceae, is the only species of the genus present in West Africa [1-4]. The plant is almost exclusively tropical, being restricted to Senegal. Nigeria and down to Angola in Central Africa [1, 2, 5, 3]. It has a representative in tropical areas [5]. Some evidence in the literature indicates that it may be one of the few trees of a truly African origin [6]. In Nigeria, the tree is restricted to the rain forest zones, especially Ijebu, Ishan Benin and Ogba in Edo State; Onitsha and Enugu, and Imo, Abia, Delta, Akwa Ibom, Crossriver and Ebonyi states [2]. This established distribution pattern indicates that the original range of the tree crop in Nigeria spanned the whole of the southern region, from the west to the east within lat. 4° 15' N and 8° N [7].

According to Onwuchi [8], P. macrophylla seeds play an important role in the diet of many people in Southeastern Nigeria. They can be processed into a variety of high protein foods [9]. It is estimated that some thirty million people from Eastern Nigeria depend on the bean oil for food, deriving about 23.04% protein and 48.56% starch from it [10]. The processed seeds are used as a delicacy and as food during cultural festivals such as weddings, new yam festivals, and 'Ibo Uzo' and 'Iwa-Akwa' ceremonies. The fermented seeds are used as seasoning in the preparation of local soups and local lettuce served to visitors, in conjunction with melon cakes. In these forms, the seeds contribute protein, calories and variety to what would otherwise be a monotonous carbohydrate-dominated diet. The dry empty pods serve as important fuel wood [3]. Allen and Allen have also reported that paucine, an alkaloid found in the seed of P. macro*phylla*, is used as an emetic and a snake-bite remedy in some primitive areas of South America. The seeds also contain iodine, acid and oil of a high melting point which can be used in soap-making [11].

The numerous uses of the multi-purpose plant seeds and pods aroused our interest in investigating the pests and mycoflora which, according to Aju [12], constitute a drawback to their production, value and use.

2. Materials and methods

The mature and ripe pods of *P. macrophylla* used for the study were collected, in August and September 2001 with a daily temperature of 29 °C, from different locations in Umuagwo, Obowo and Mbaise in Imo State of Nigeria. The pods were harvested from the tree-tops using a cutlass. The pods were packed in sterile containers and polvethylene bags. The matured dry and fresh pods, which could be identified by their brown and white seeds, respectively, were opened along the longitudinal slits using a cutlass. Samples were stored in polyethylene bags and placed in the laboratory before being used for the study. The pods were not treated with any pesticide or fungicide before storage.

To identify the pests on the tree-tops, observations were made of about 20 pods on five trees in each of the three areas; they were stored in the laboratory. Twenty pods from each of the five areas were also opened using a cutlass and the larvae of the insect pests were counted and reared to maturity in collecting bottles with holes in the cork for air. For identification, photographs of intact and opened pods were taken with a Lubitel 2 camera. The stored pods were periodically sampled for pests and mycoflora.

Isolation, characterization and identification of the mycoflora were done following the method of Okigbo [13]. Washings from the pods, skin and seeds were used. Five serial dilutions of the washings were prepared using sterile peptone water, and were plated on yeast malt extract agar (Difco) and potato dextrose agar (PDA). The plates were incubated at 28 °C for (2 to 3) days. Identification of the mycoflora was done using standard procedures [14, 15]. Control plating was done with sterile peptone water. In both the test and control media, streptomycin was incorporated to avoid bacteria.

For the sake of purity, colonies were picked from the incubated plates and subcultured on yeast malt extract agar and potato dextrose agar.

For the pathogenicity test, the method of Okigbo [13] was followed. Intact pods and seeds of *P. macrophylla*, which were surface-sterilized with 70% alcohol and cleaned

Table I.

Insect pests found on the pods and seeds of Pentaclethra macrophylla collected on tree-tops (Nigeria) [^(r): regular pests and ^(o): occasional pests].

Order	Common name	Scientific name	Part of pod or seed attacked
Coleoptera Hemiptera	Snouted beetle (pod/seed borer) Black capsid Brown capsid Brownshield bug	Sitophilus sp. ^(r) Distantiella theobroma ^(o) Sahlbergella singularis ^(o) Pathycoelia thalassoma ^(o)	Grub bore into pods and feed on the seeds Feed on flower and young pods Feed on young pods. Feed on young pods
Hymenoptera Lepidoptera	Hemipterous shield bug Ant Stem / pod borer Armyworm or geometrid (caterpillar) Silkworm	Nezara sp. ^(o) Formica sp. ^(o) Cossus cadaribae ^(r) Spodoptera exempta ^(r) _ ^(r)	Feed on young pods Feed on young and tender pods Larvae bore into pods and feed on seeds Larvae skelerotonize young pods Larvae eat into the pods, feeding on the pods and seeds
	Pod and seed borer	Characoma stictigrapta	Bore into the pods and feed on seeds

with distilled water, were used. The fungi used for the test were Botrvodiplodia theobromae, Aspergillus niger and Rhizopus stolonifer. Fungus spore suspensions were prepared by centrifuging and resuspending in three changes of sterile distilled water. The surface of the pods, that had already been lacerated with a sterile scalpel, was spread with the spore suspension using a rocking sprayer. The sprayed pods were covered with sterile transparent cellophane sheets and kept in a safe place. They were examined daily to detect disease symptoms.

3. Results

Ten pests were implicated in the attack of the pods and seeds of P. macrophylla on the tree-top. Among them, Cossus cadaribae, Sitophilus sp., the silkworm and Spodoptera exempta were most frequently found, since they occurred in the samples collected from the three areas. These pests have been designated as regular pests (table I). Larvae of Cossus cadaribae and the snouted beetle Sitophilus sp. were mostly found in the lesions in the seeds (figure 1) and were associated with the observed damage. The silkworms were found restricted to the pods in small pockets (figure 2) while the geometrids were observed feeding on young pods.

The other pests, Distantiella theobroma, Sahlbergella singularis, Pathycoelia thalassoma, Nezara sp. and Formica sp., were found in only about 50% of the sampling locations: they were observed feeding on young pods and flowers, causing fruit drop and malformation of the pods.

In storage, the dominant pests found in some of the pods and seeds were Araecerus fasciculatus, feeding on the fresh cotyledons, Callosobruchus maculatus on dry seeds and Sitophilus sp. Adults of this last pest were found in cracks on the pods and larvae were located in lesions of the seeds covered by some tough silk-like materials (table II). Plodia interpunctella and Ephestia cautella were occasionally found. Larvae of *P. interpunctella* fed on dry pods leaving Figure 1. silken threads and droppings, and pupae of E. cautella were found in cocoons on the macrophylla attacked by larvae pod surface. *Microtermes* species found in of pests and moulds.

Seeds of Pentaclethra





Figure 2. Pods of *Pentaclethra macrophylla* affected by pests and moulds. two specimen groups fed voraciously on the dry pods, while millipedes, which are not insects, were found in the group which had been exposed to the termites.

The major species of mould (with more than 20% incidence) found on the surface of intact pods was *Aspergillus niger*, while the minor moulds were *Aspergillus sp.* and *Rhizopus stolonifer (table III)*. The moulds found on the surface and inside wall of the pods with cracks, wounds and different shades of discoloration (*figure 2*) were *Aspergillus niger, Rhizopus stolonifer, Fusarium* sp., and *Penicillium* sp., respectively, while the minor moulds were *Botryodiplodia theobromae, Rigidoporus lignosus* and *Cladosporum herbarum*.

The surface of intact seeds had *Aspergillus niger* as the major mould and *Aspergillus* sp., *R. stolonifer* and *Mucor* sp. as minor

moulds (*table III*). Damaged and rotted seeds (*figure 3*) had *B. theobromae, C. herbarum, Fusarium* sp., *A. niger* and *Mucor* sp. as major mould species.

The results of the pathogenicity test showed enhanced necrotic lesions on the pods; the fungus re-isolated from the affected pods was mainly *B. theobromae*. The seeds sprayed with spore suspensions of *Fusarium* sp. and *Aspergillus niger* showed rotting at a later stage.

4. Discussion

The importance of pests and diseases as one of the factors causing shrinkage in the distribution and production of *P. macrophylla* trees in Southeastern Nigeria has been stressed by Aju [12]. Nevertheless, a study on the pests and diseases of the pods and seeds of this multi-purpose tree crop has not been well documented. However, pods and seeds are seriously attacked by pests and diseases, judging from the degree of damage of the pods (*figure 2*) and seeds (*figure 1*) noticed in this study.

Within the limits of the precautions taken in our experiments, it can be concluded from our results that the major pests of *P. macrophylla* include *Cossus cadaribae*, *Sitophilus* sp. and armyworms, which cause debilitation effects, such as slow growth, stunting and, in severe cases, death of young pods. On mature pods, these pests cause holes and cracks (*figure 2*), which constitute a portal through which moulds penetrate to rot the seeds (*figure 1*). Their larvae equally feed on and damage the

Table II.

Pests founds on the pods and seeds of *Pentaclethra macrophylla* after their storage in the laboratory (Nigeria) [^(r): Regular pests and ^(o): occasional pests].

Order	Common name	Scientific name	Part of pod or seed attacked
Coleoptera	Bean weevil Snouted beetle	Araecerus fasciculatus ^(r) Sitophilus sp. ^(r)	Adults and larvae feed on the cotyledons Adults found in cracks on the pods. Larvae feed on seeds
	Pulse beetle	Callosobruchus maculates ^(r)	Adults feed on dry seeds
Lepidoptera	India meal moth	Plodia interpunctella ^(o)	Larvae feed on dry pods, leaving silken threads and droppings
	Warehouse moth	Ephestia cautella ^(o)	Pupae found in cocoon on pod surface
Isoptera	Termites	<i>Microtermes</i> sp. ^(o)	Feed voraciously on the dry pods
Myriapoda	Millipedes	_(o)	Found in the heap of pods

Table III.

Mycoflora found on various parts of pods and seeds of Pentaclethra macrophylla.

Part of pods or seeds	Mycoflora incidence		
	Major (20–100%)	Minor (< 20%)	
Outer surface of pods without cracks or wounds	Aspergillus niger	Aspergillus sp., Rhizopus stolonifer	
Inner surface of pods without crack or wounds	Aspergillus niger	· _	
Outer surface of pods with cracks, wounds and	Aspergillus niger,	_	
discolored parts	Rhizopus stolonifer.		
	Fusarium sp.,		
	Penicillium sp.		
Inner surface of pods with cracks, wounds and	Asperaillus niaer.	Botrvodiplodia theobromae.	
discolored parts	Rhizopus stolonifer.	Riaidoporus lianosus.	
	Fusarium sp.	Cladosporum herbarum	
	Penicillium sp	0.4400p0.4	
Surface of intact seed	Asperaillus niger	Asperaillus sp.	
	, iopolginao ingol	Rhizopus stolonifer	
		Mucor sp	
Damaged and rotted seed	Botrvodiplodia theobromae	-	
Damaged and rotted beed	Cladosporium herbarum		
	Fusarium son		
	Asperaillus niger		
	Aspergillus Iligei		

seeds (*figure 3*). The minor pests also cause damage by feeding on the young pods.

In storage conditions, *Araecerus fasciculatus* and *Callosobruchus maculatus* feed on the cotyledons of the seeds, damaging and reducing their value, while *Plodia interpunctella, Ephestia cautella* and *Microtermes* sp. concentrate on the pods. Surprisingly, both the tree-top and storage pests are members of only five orders, namely, Coleoptera, Hemiptera, Hymenoptera, Isoptera and Lepidoptera.

The mycoflora included Aspergillus sp., Aspergillus niger, Rhizopus stolonifer, Fusarium sp., Penicillium sp., Botryodiplodia theobromae, Cladosporum herbarum and Mucor sp. Most of the moulds occurred on the outer surface and inner surface of cracked and damaged walls of the pods and on the damaged and rotted seed. The discoloration of the surface of the pods with cracks and wounds (figure 2) and the rotting of the seeds as a result of mould were a result of infection. The implication of bacteria is subject to further investigation. It has been suggested that the moulds implicated in the seeds could have entered through wounds and cracks. The mode of their entry, however, needs to be investigated further. Pests of P. macrophylla other than insect pests

need to be surveyed and identified, including its bacterial pathogens.

Acknowledgements

I wish to thank professor M.C. Eluwa, department of Biological Sciences (zoology), for identifying some of the insect pests

Figure 3.

Opened pods of *Pentaclethra macrophylla* showing pest and mould damages.



and M. Shola, department of microbiology, for his assistance with the culture isolation and identification of the mycoflora. Prof. H.O. Edeoga is also acknowledged for his advice, as is G.G.E. Osuagwu for reading through the manuscript.

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Insectos parásitos y micoflora de las vainas y semillas de *Pentaclethra macrophylla* Benth. en el sudeste de Nigeria.

Resumen — Introducción. Pentaclethra macrophylla, Mimosaceae, es la única especie del género presente en África occidental. No se conocen bien las enfermedades de esta planta. Los numerosos usos de las vainas y semillas del árbol nos llevaron a estudiar los parásitos y la micoflora de estos elementos, que supondrían un obstáculo para la producción de la especie, su valorización y su utilización. **Material y métodos**. Se recogieron algunas vainas maduras de *Pentaclethra macrophylla* en distintas zonas de Nigeria, efectuándose también algunas observaciones de la parte superior del árbol para estudiar los parásitos presentes. El aislamiento, la caracterización y la identificación de la micoflora se efectuaron mediante inoculación del agua de aclarado de las vainas, de la piel y las semillas en distintos medios incubados durante 2 a 3 días a 28 °C. La inoculación testigo se hizo en agua de peptona estéril. En los medios de cultivo de ensayo y testigo se añadió estreptomicina para evitar el desarrollo de bacterias. Al mismo tiempo, se almacenaron algunas vainas y semillas en laboratorio antes de realizar el tratamiento citado. Se efectuó también un ensayo de patogenicidad. Resultados y discusión. Se encontraron muchas especies de insectos y microbios que atacaban las vainas y semillas de las plantas. Los principales insectos parásitos identificados fueron *Cossus cadaribae*, *Sitophilus* spp., *Spodoptera exempta* y gusanos de seda. Los principales patógenos fúngicos fueron *Aspergillus niger*, *Rhizopus stolonifer*, *Cladosporium* herbarum, Penicillium sp., Botryodiplodia theobromae y Fusarium sp. Algunos de los insectos parásitos destruyen las vainas verdes privándolas de sus superficies fotosintéticas; algunos taladran las vainas al alimentarse y dañan los cotiledones carnosos de las semillas, otros desgarran las vainas provocando lesiones de diferentes tamaños y formas que constituyen entradas para la penetración de patógenos fúngicos y bacterias que invaden las vainas y descomponen las semillas accesibles. Conclusiones. Nuestro estudio mostró que muchos parásitos y elementos de la micoflora atacaban las vainas y las semillas de Pentaclethra macrophylla. Podrían ser la causa de los síntomas y daños observados y explicar la regresión de la planta cultivada, relatada en anteriores estudios.

Nigeria / Pentaclethra macrophylla / plagas / flora microbiana / identificación