Global Journal of Science Frontier Research: C
Biological Science
Volume 15 Issue 4 Version 1.0 Year 2015
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Gmelina Arborea Roxb: Associated Mycoflora and Diseases in Cross River State, Nigeria

By Umana, E. J, Akwaji, P. I., Markson, A. A & Udo, S. E
University of Calabar, Calabar, Nigeria

Abstract- *Gmelina arborea* Roxb is a major income earner for the Government and people of Cross River State, Nigeria. In spite of the vast economic potential of this plant in Nigeria, very little pathological research had been done on diseases afflicting this valuable species. In view of this, a survey on mycoflora and diseases associated with G. *arborea* was carried out in major plantations in Oban and Awi in Akamkpa Local Government Area and Ovonum in Obubra Local Government Area of Cross River State, Nigeria. Twenty four plant stands were sampled in four sites per location. The plant stands were examined for various disease symptoms and the means of infection calculated in each location. Percentage frequencies (occurrence) were also determined using the means. Randomized Block Design (RBD) was used for the experiment. A total of twenty fungi were isolated (root, bark, leaf, seed and soil) while five fungal diseases were identified in the field. The fungal isolates were: *Aspergillus flavus, Aspergillus niger, Apodachlya pyrifera, Botroyodiplodia theobromae, Bouvularia sp, Ceratocystis fimbriata, Cercospora appi, Chalaropsis sp, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Penicillium thomii, Phoma herbarum, Rhizopus stolonifer, Thielaviopsis brasicola, Trichoderma viride, Trichosporonoide oedocephalus* and *Graphium penicilliodes*. The diseases were leaf spot, stem canker, die back, Butt and root rot and Damping off.

Keywords: *gmelina arborea roxb, mycoflora, diseases, cross river state, nigeria.*

GJSFR-C Classification : *FOR Code: 270499*

Strictly as per the compliance and regulations of :

Gmelina Arborea Roxb: Associated Mycoflora and Diseases in Cross River State, Nigeria

Umana, E. J [α], Akwaji, P. I. [σ], Markson, A. A [ρ] & Udo, S. E [ω]

Abstract- *Gmelina arborea* Roxb is a major income earner for the Government and people of Cross River State, Nigeria. In spite of the vast economic potential of this plant in Nigeria, very little pathological research had been done on diseases afflicting this valuable species. In view of this, a survey on mycoflora and diseases associated with G. *arborea* was carried out in major plantations in Oban and Awi in Akamkpa Local Government Area and Ovonum in Obubra Local Government Area of Cross River State, Nigeria. Twenty four plant stands were sampled in four sites per location. The plant stands were examined for various disease symptoms and the means of infection calculated in each location. Percentage frequencies (occurrence) were also determined using the means. Randomized Block Design (RBD) was used for the experiment. A total of twenty fungi were isolated (root, bark, leaf, seed and soil) while five fungal diseases were identified in the field. The fungal isolates were: *Aspergillus flavus, Aspergillus niger, Apodachlya pyrifera, Botroyodiplodia theobromae, Bouvularia sp, Ceratocystis fimbriata, Cercospora appi, Chalaropsis sp, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Penicillium thomii, Phoma herbarum, Rhizopus stolonifer, Thielaviopsis brasicola, Trichoderma viride, Trichosporonoide oedocephalus* and *Graphium penicilliodes*. The diseases were leaf spot, stem canker, die back, Butt and root rot and Damping off. Of the twenty fungal isolates, A. *flavus* occurred in all parts of the plant and the soil while others occurred in four or three parts. Pathogenicity tests revealed that all the isolated fungi were pathogenic on G. *arborea*.

Keywords: gmelina arborea roxb, mycoflora, diseases, cross river state, nigeria.

I. Introduction

Gmelina arborea Roxb (Family: Verbanaceae) is reported to be widely grown deciduous tree of moderate to large size with an arborescent habit hence the specific name "*arborea*" (Cromer *et al.,*1993). It is a fast growing tree, which grows faster than some exotic species under the same conditions. It is medium-sized, reaching a height of about 30 – 40m, with a bole averaging 40cm in diameter but sometimes attaining 50cm. The leaves are more or less heart-shaped, 10 - 25cm × 5 - 18cm and globrous or velvety beneath, the corolla is bright yellow and the ovary glabrous (Anon, 2002). Drupes are ovate or pyriform, 2 - 2.5cm long, smooth, becoming orange-yellow, pulpy with large egg-shaped stone, having 1 - 4cells, seeds 1 - 4 (Duke, 2002). The drupes are reported to contain butyric acid, traces of tartaric acid resinous and saccharine matter. Resinous and saccharine matter and benzoic acid are also found in roots (Julian, 1982).

Gmelina arborea is native to tropical moist forest from India, Burma and Sri Lanka to Southern China. It is widely introduced in Brazil, Gambia, Honduras, Ivory Coast, Malaysia, Malawi and Sierra Leone (Duke, 2002). Ademiluyi and Okeke (1973) described *G. arborea* as one of the widely grown plantation species in Nigeria. Best development of *G.arborea* in Nigeria occurs where air temperature ranges from 18°C - 35°C, with distinct dry season, and relative humidity above 40%. The occurrence of these climatic features in West Coast of Africa accounts for the success of *G. arborea* in Nigeria, Cote d'Ivoire, Sierra Leone and Ghana (Chijoke, 1986, FAO, 1989). *G. arborea* can be propagated by seeds or cuttings (wildings and root cuttings) (Enemuoh, 1970).

Gmelina arborea Roxb is an economic tree with vast uses as timber and is a major source of raw material for the construction, instrument and paper industry (Duke, 2002). *G. arborea* timber is reasonably strong for its weight. It is used in constructions, furniture, carriages, sports, musical instruments and artificial limbs. Once seasoned, it is a very steady timber and moderately resistant to decay and ranges from very resistant to moderately resistant to termites. Its timber is highly esteemed for door and window panels, joinery and furniture especially for drawers, wardrobes, cupboards, kitchen and camp furniture, and musical instruments because of its light weight, stability and durability. It is also used for bentwood articles. In boat building it is used for decking and for oars. *G. arborea* is a popular timber for picture and slate frames, turnery articles and various types of brush backs, brush handles and toys also for handle of chisels, files, saws, screw drivers, sickles etc. The wood is also used for manufacturing tea chests and general purpose plywood, blackboards, frame core and cross bands of flush door shutters. In the instrument industry *G. arborea* timber is widely employed for the manufacture of drawing boards, plane tables, instrument boxes, thermometer scales and cheaper grade metric scales. It is also used in artificial limbs, carriages and bobbins. It is an approved timber for handles of tennis rackets, frames and reinforcements of carom boards and parking cases and crates. *G. arborea* is used in paper making and matchwood industry. *G. arborea* leaves are considered good for

Author α σ ρ: Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria. e-mail: akwajiisnever@yahoo.com
Author ω: Department of Biological Sciences, Cross River University of Science and Technology, Calabar, Cross River State, Nigeria.

cattle (crude protein -11.9%). The root and bark of *G. arborea* are claimed to be stomachic, galactagogue laxative and anthelmintic, improve appetite, useful in hallucination, piles, and abdominal pains, burning sensations, fevers and urinary discharge. Leaf paste is applied to relieve headache and juice is used as wash for ulcers. Flowers are sweet, cooling, bitter, acrid and astringent. They are useful in leprosy and blood diseases (Agu *et al.,* 2002, Duke, 2002, Anon, 2002, Haygreen and Bonyer, 1982, Ogbonnnaya *et al.,* 1992, Greaves, 1973).

The fungal pathogens which have been implicated in the pathogenesis of *G. arborea* include *Fomes lignosus* and *Heterobasidium annosum* that cause butt and root rot of this plant (Inyang, 1990). Disease caused by *Ceratocystis fimbriata* is more severe in moist climates. *Poria rhizomorpha* causes stem and root diseases in wet situations. Duke (2002) mentioned the following as the fungi affecting *Gmelina arborea*: *Armillaria mellea*, *Cercospora ranjita*, *Fomes roseus*, *Poliporus baudni*, *Poria rhizomorpha* and *sclerotina rolfsii*. Most fungal attack occurs at the seedling stage, which is very delicate stage of growth of this plant. Julian (1982) reported that a whole bed of seedlings could be affected by fungal diseases. The types of fungal attack include damping-off, moulds and stem rot. Damping-off is common among seedlings in the tropics, the tissues rot near the collar causing death of the tree. The main symptom is toppling-over caused by fungi in the following genera: *Fusarium*, *Pythium*, *Rhizoctonia*, *Penicillium* and *Phytophthora*, causing about 50% seedling mortality rates if unchecked (Julian, 1982). Mould is a foliage disease affecting seedling of any size and is generally caused by species of *Botrytis*, *Penicillium* and other fungi. The attack of these diseases is aggravated by cooler and moister conditions. The fungal attacks are not only limited to the seedling stages, the bigger plant in the field can be affected. All parts of the plant can be affected by fungal diseases (Duke, 2002). In spite of the vast economic potential of this plant in Nigeria, very little pathological research had been done on diseases afflicting this valuable species. In view of this, an extensive investigation on the mycoflora and diseases associated with *G. arborea* was carried out across major *G. arborea* plantations in Cross River State, Nigeria.

II. Materials and Methods

a) Sources of materials

The diseased plant parts comprising of the fruits, roots, stems and leaves were collected from different and widely spread locations in Cross River State, Nigeria namely Awi and Oban *Gmelina* plantations in Akamkpa Local Government Area and Ovonum in Obubra Local Government Area. Wildlings and seeds for planting (used for pathogenicity test) were collected from *G. arborea* plantation in Awi, Akamkpa L.G.A. Soils were also collected from the rhizosphere of the plantation from the three locations. The soils were put in polyethylene bags. Soil sample (sandy - loam) was also obtained from Ovonum in Obubra L.G.A, Cross River State, Nigeria for soil analysis. The Laboratory work was carried out in the Laboratory and Green house of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

b) Survey of diseases of G. arborea

The study was conducted in three localities, Awi and Oban in Akamkpa L.G.A and Ovonum in Obubra L.G.A of Cross River State, Nigeria. Twenty four plant stands were sampled in four sites per location. The plant stands were examined for various disease symptoms and the means of infection (Disease incidence) calculated in each location, percentage infections were also determined using the means. The data collected were subjected to statistical analysis at $p < 0.05$. Randomized Block Design (RBD) was used for the experiment. Disease incidence was calculated using the formula:

$$\text{Disease incidence (I)} = \frac{\text{Number of infected plant units} \times 100}{\text{Total number (healthy and infected of units assessed)}}$$

c) Isolation and identification of fungi associated with G. arborea

Isolations were made from diseased plant parts (leaf, stem, fruit, and root) and rhizosphere (soil). This was carried out using the method of Richter and Dallwitz (2000). Pieces of the diseased parts were cut with a sterile scalpel and placed separately. These were then later washed several times with distilled water and sterilized with 95% ethanol. Sterile inoculating needle was used to pick the parts and placed on Potato Dextrose Agar (PDA), then incubated for seven days at 28 ± 1°C. These were sub -cultured until pure cultures were established for identification. Isolation from soils was carried out by dilution plate and soil washing method as described by Halverson *et al.,* (1993) and Tsao (1983). For soil dilution plate method, 20grams of soil from the different locations (*Gmelina arborea* plantations) were collected in polyethylene bags. Ten grams (10g) of each soil was suspended in 90ml of distilled water. Ten-fold dilution series was made and 1ml of each was incorporated into PDA in Petri dishes (9mm). The plates were incubated at room temperature 28 ± 1°C for seven days and fungal counts made from 10^{-1} dilution plates and recorded in percentages. For soil washing method, ten grams (10g) of each soil sample were separately suspended in 90ml of sterile distilled

water. The supernatant was in each case decanted and the settled particles washed into 250ml flasks with 50ml of sterile distilled water. The soil was shaken and allowed to settle at an angle of 45˚C for five minutes, and then the liquid was again decanted. The washing was repeated five times after which 1gram of the soils was each aseptically transferred into Petri dishes (9mm) containing 20ml of molten PDA and incubated at 28 ± 1˚C for seven days. The percentage occurrence of each fungus was recorded and the pure culture of each prepared by aseptically transferring the mycelia to newly prepared PDA and incubated at 28 ±C1 for seven days. Fungi identification was carried out by microscopic studies of the isolates. Identification of the isolates were based on morphological characteristics, described in (1998) Illustrated Genera of Fungi by Barnett and Hunter and with literature on the Identification of Pathogenic Fungi by Dugan (2006). Confirmation was made by comparing with cultures identified by International Mycological Institute, Egham, UK.

The frequencies of occurrence of the isolated fungi associated with different diseases (parts) of *G. arborea* were also determined. The number of time each fungus was encountered was recorded. The percentage frequency of occurrence was calculated using the formula:

$$\frac{\text{Number of times a fungus was encountered} \times 100}{\text{Total fungal isolations}} \quad \text{(Ebele, 2011)}$$

d) Soil Analysis

Sandy-loam soil collected from Ovonum in Obubra L.G.A of Cross River State, Nigeria used for the planting of *G. arborea* was analyzed at the Research Laboratory, Department of Soil Science, University of Calabar, Nigeria for percentage moisture, pH, total Nitrogen N (determined using Kjedahl's method followed by spectrophotometry procedure), organic carbon (determined by oxidation with $K_2Cr_2O_7$, Yeoman's and Bremner, 1998, Available phosphorus P (determined using the method of Murphy and Riley, 1972), Potassium K (determined using flame photometry).

e) Soil sterilization/planting of G. arborea

Soil sterilization was conducted in the Department of Botany green house, University of Calabar, Nigeria under mean temperature of 30̊C. The top soil collected at 0-40cm depth were heat sterilized in a cut covered metal drum using firewood at 100˚C for 20 minutes and allowed to cool. The sterilized soil was dispensed into polyethylene bags. The polyethylene bags were filled with about 5 kilogram (5kg) of the sterilized sandy-loam soil and *G. arborea* seeds and seedlings obtained from the wild sown on the soil. Two to three seeds of *G. arborea* were sown on the sterilized soil. After seedling emergence, they were thinned down to one stand.

f) Koch's Postulates and Pathogenicity Test (Disease severity)

To confirm pathogenicity of fungal isolates obtained from (leaf, stem, root, bark and soil), a seven-day old culture of isolates filtrates from the different location were grown on basal medium supplemented with pectin as the only carbon source. This was done by pouring 100ml of inoculums at the base of the plants. The plants were earlier wounded with a sterile inoculating needle to facilitate entry of spores (Koleosho *et al.,* (1987). The base of the plant stands were then covered with polyethylene bags for one day to prevent moisture loss and entry of other pathogens. Pathogenicity tests were carried out at intervals and in sets when the plants were 4 months, 8 months and 12 months old. On appearance of symptoms, the area of infection was measured (determined) using a metre rule (mm) and the mean percentage infection (Disease severity) calculated using the formula:

$$\text{Disease severity (S) (Area)} = \frac{\text{Area of plant tissue affected}}{\text{Total area}}$$

Pathogenicity tests of each isolate were replicated thrice.

III. Results

a) Fungal diseases of G. arborea identified in the field

A total of five fungal diseases were identified in the field (Awi, Oban and Ovonum) during the survey. The diseases were leaf spot, stem canker (Plate 1), Die bark (Plate 2), Butt and root rot and Damping-off (Table 1). Out of the three locations sampled, Awi and Ovonum had 12 stands each being infected by leaf spot disease while 18 stands were infected in Oban which represented 50% and 75% infection respectively. Stem canker was observed on 10 stands (41.67%) in Awi, 20 stands (83.3%) in Oban and 12 stands (50%) in Ovonum. Damping-off disease was the most observed disease in the three locations with a total of 15 stands (62.5%) in Awi, 18 stands (75%) in Oban and 16 stands (66.67%) in Ovonum. The results showed that all the diseases were more prevalent in Oban than Awi and Ovonum (Table 1).

Table 1 : Number of sampled and infected plant stands by the various fungal diseases of *G. arborea* in Awi, Oban and Ovonum.

Diseases	Total number of plant samples	Number of infected plants		
		Awi	Oban	Ovonum
Leaf spot	24	12.12 ± 0.04	18.0 ± 0.08	12.1 ± 0.06
Stem canker	24	10.12 ± 0.07	20.14 ± 0.02	12.03 ± 0.05
Die-bark	24	7.01 ± 0.11	13.12 ± 0.10	10.12 ± 0.07
Butt and root rot	24	13.11 ± 0.06	17.10 ± 0.09	12.4 ± 0.09
Damping off	24	15.06 ± 0.08	18.1 ± 0.08	16.10 ± 0.5

Note: Values are means of three replicates ± standard error.

Plates 1 & 2 : Photographs of Stem canker and Die bark disease of *G. arborea* caused by fungi as observed in Awi, Oban and Ovonum.

b) Fungal isolates of different parts of G. arborea and soil

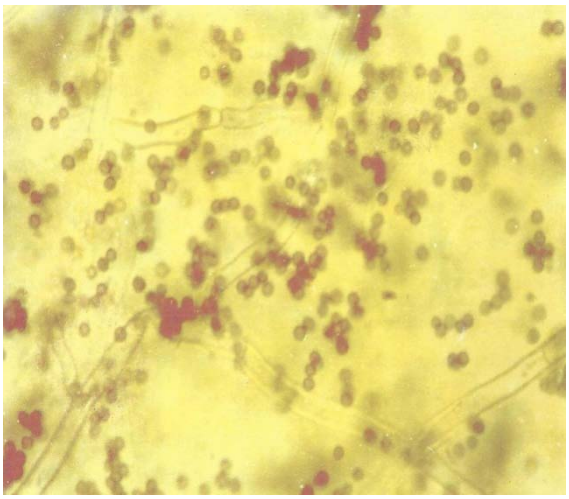
A total number of twenty fungi were isolated from different parts (root, bark, leaf and seed) of *G. arborea* as well as soil. A breakdown of the isolated fungi is presented in (Table 2).

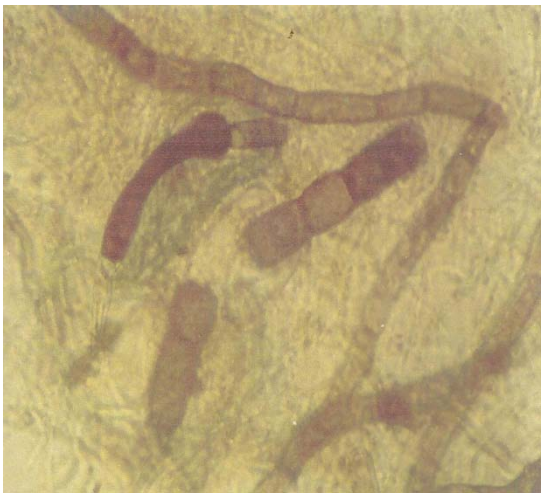
Table 2 : Fungal isolates as sociated with different parts of *G. arborea* and soil

S/N	Fungi	Source of inoculum				
		Root	Bark	Leaf	Seed	Soil
1.	*Aspergillus flavus* Link	✓	✓	✓	✓	✓
2.	*Aspergillus niger*. Van Tiegh	✓	✓		✓	✓
3.	*Apodachlya pyrifera*		✓	✓	✓	✓
4.	*Botryodiplodia theobromae*. Pat	✓	✓			✓
5.	*Bouvularia sp*			✓	✓	
6.	*Ceratocystis fimbriata*. Ellis		✓			✓

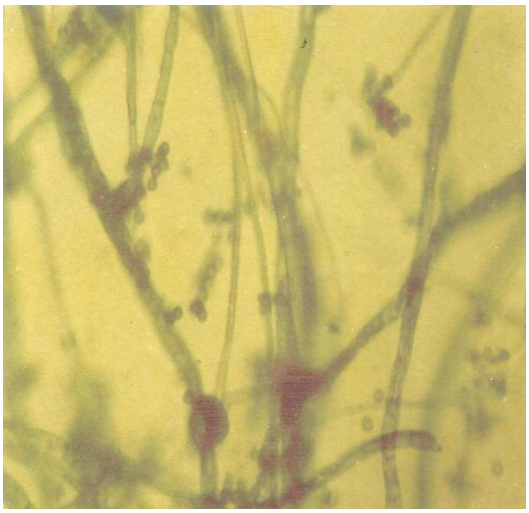
7.	*Cercospora appi*. Fresen	✓			✓	✓	
8.	*Chalaprosis sp*. Peyron				✓	✓	✓
9.	*Dacromyces deliquescense*	✓	✓				✓
10.	*Fusarium oxysporum*. Link: Fr.	✓	✓				✓
11.	*Geotrichum sp* Link: Fr.	✓			✓	✓	✓
12.	*Mucor mucedo*. Link: Fr.	✓				✓	
13.	*Penicillium vermiculatum* Link: Fr.	✓				✓	
14.	*Penicillium thomii*.Link: Fr.	✓				✓	✓
15.	*Phoma herbarum*. Sacc		✓		✓		
16.	*Rhizopus stolonifer*. Ehrenb: Fr.	✓			✓	✓	✓
17.	*Thielaviopsis brasicola*: Berk and Broom	✓					✓
18.	*Trichoderma viride*. Pers: Fr.					✓	✓
19.	*Trichosporonoide oedecephalos*		✓				
20.	*Graphium penicilliodes*	✓	✓			✓	✓

Results (Table 2) shows that *Aspergillus flavus* was isolated from infected root, bark, leaf and seed of *G. arborea,* while *A. niger* was isolated from infected root, bark, seed and soil. *Apodachlya pyrifera* (Plate 3) was isolated from infected (bark, leaf, seed and soil), *Botryodiplodia theobromae* (Plate 4) (root, bark and soil), *Bouvularia sp* (leaf and soil), *Ceratocystis fimbriata* (bark and soil), *Cercospora appi* (root, leaf and seed), *Chalaropsis sp* (leaf, seed and soil), *Dacromyces deliquescens* (Plate 5) (root, bark and soil), *Geotrichum sp* (Plate 6) (root, leaf, seed and soil), *Mucor mucedo* (Plate 7) (root and seed), *Fusarium oxysporum* (Plate 8) (root, bark and soil), *Penicillium vermiculatum* (root and seed), *Penicillium thomii* (root, seed and soil), *Phoma herbarum* (bark and leaf), *Rhizopus stolonifer* (root, leaf, seed and soil), *Thielaviopsis brasicola* (Plate 9) (root and soil), *Trichoderma viride* (seed and soil), *Trichosporonoide oedocephalos* (Plate 10) (bark) and *Graphium penicilloides* (root, bark, seed and soil).

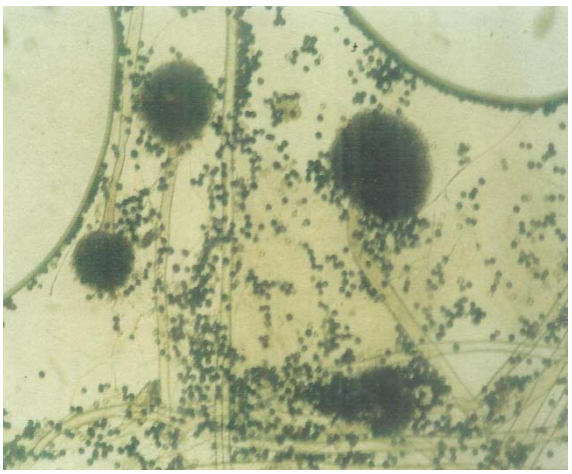


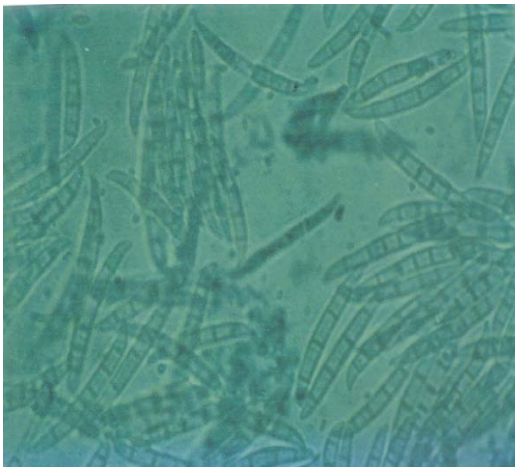


Plates 3 & 4 : Photomicrographs of *Apodachlya pyrifera* and *Botryodiplodia theobromae* × 400 isolated from infected *G. arborea* parts and rhizosphere (soil)

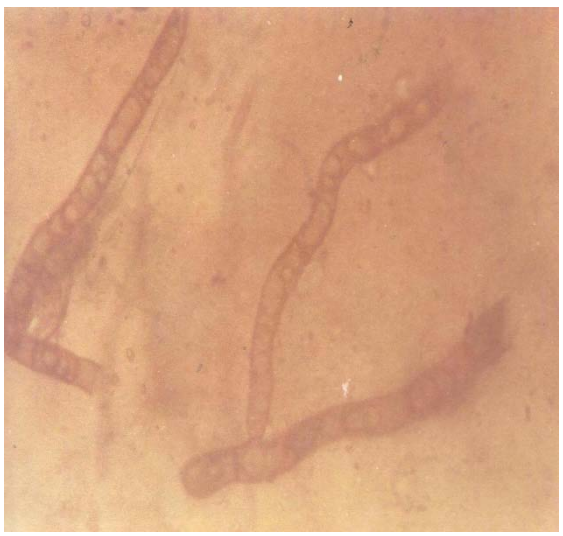


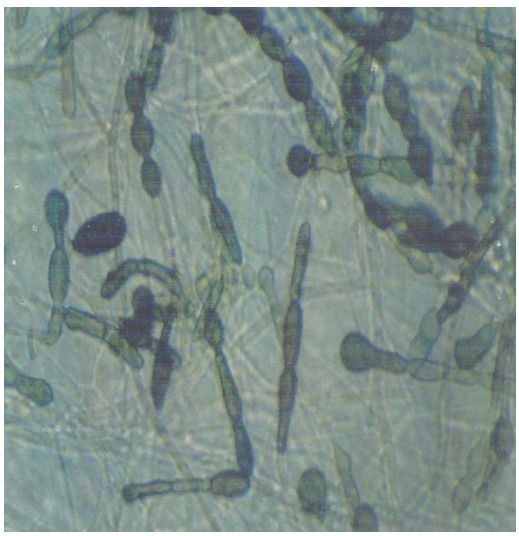
Plates 5 & 6 : Photomicrographs of *Dacromyces deliquescens* and *Geotrichum sp* × 400 isolated from infected *G. arborea* parts and rhizosphere (soil)





Plates 7 & 8 : Photomicrographs of *Mucor mucedo* and *Fusarium oxysporum* × 400 isolated from infected *G. arborea* parts and rhizosphere (soil)





Plates 9 & 10 : Photomicrographs of *Thielaviopsis brasicola* and *Trichosporonoide oedocephalos* × 400 isolated from infected *G. arborea* parts

c) *Percentage frequency (occurrence) of fungal isolates*

Mean percentage frequencies (occurrence) of fungi isolated from different parts of *G. arborea* and soil is presented in (Table 3). Prominent among the fungi isolated from root were *Botryodiplodia theobromae* with mean percentage frequencies of 11.00 ± 0.10% followed by *Aspergillus flavus* and *Thielaviopsis brasicola* with mean percentage frequencies of 9.55 ± 0.39% each. *Apodachlya pyrifera*, *Bouvularia sp*, *Chalaropsis sp*, *Phoma herbarum*, *Trichoderma viride* and *Trichosporonoide oedecephalos* were not present in the infected root of *G.arborea*.

The prominent fungi isolated from the infected bark of *G. arborea* were *Apodachlya pyrifera*, *B. theobromae* and *Graphium penicilloides* with mean percentage frequencies of 13.76 ± 0.42% each, followed by *Fusarium oxysporum* 12.84 ± 0.32%, *Ceratocystis fimbriata* and *Dacromyces deliquiscens* with mean frequencies of 11.93 ± 0.06% each. However, the following fungi *Bouvularia sp*, *Cercospora appi*, *Chalaprosis sp*, *Geotrichum sp*, *Mucor mucedo*, *Pernicillium vermiculatum*, *Penicillium thomii*, *Rhizopus stolonifer*, *Thielaviopsis brasicola* and *Trichoderma viride* were absent in the bark of *G. arborea*.

The highest mean percentage frequencies of fungi isolated from the leaf of *G. arborea* were that of *Aspergillus flavus*, *Cercospora apii* and *Rhizopus stolonifer* with 14.29 ± 0.49% each, followed by *Geotrichum sp* 13.19 ± 0.49%, *Aspergillus niger*, *Botryodiplodia theobromae*, *Ceratocystis fimbriata*, *Dacromyces sp*, *Fusarium oxysporum*, *Mucor mucedo*, *Penicillium vermiculatum*, *Penicillium thomii*, *Thielaviopsis brasicola*, *Trichoderma viride*, *Trichosporonoide oedecephalos* and *Graphium penicilloides* were not present on the leaf of *G. arborea*.

Aspergillus flavus, *Rhizopus stolonifer* and *Geotrichum sp* had the highest mean frequency of 8.82 ± 0.42% each from the fruit. These were followed by *Mucor mucedo*, *Penicillium thomii* and *Trichoderma viride* with mean percentage frequency of 8.24 ± 0.39% each. *Botryodiplodia theobromae*, *Ceratocystis fimbriata*, *Dacromyces sp*, *Fusarium oxysporum*, *Phoma herbarum*, *Thievaliopsis brasicola* and *Trichosporonoide oedecephalos* were absent in fruit. For soil, *Botryodiplodia theobromae*, *Fusarium oxysporum* and *Mucor mucedo* were the most prominent fungi with mean percentage frequency of 10.17 ± 0.48% each, these were followed by *Ceratocystis fimbriata* and *Geotrichum sp* with mean percentage frequency of 9.60 ± 0.45% each. The fungi that were absent from the soil are *Bouvularia sp*, *Cercospora appi*, *Penicillium vermiculatum*, *Penicillium thomii* and *Trichosporonoide oedecephalos*.

Table 3 : Percentage frequency of fungi isolated from different parts of *G. arborea* and soil

S/N	Fungi	Source of inoculum				
		Root	Bark	Leaf	Fruit	Soil
1.	*Aspergillus flavus*	9.55 ± 0.39	9.17 ± 0.41	14.29 ± 0.49	6.82 ± 0.42	0
2.	*Aspergillus niger*	8.28 ± 0.14	9.17 ± 0.41	0	7.65 ± 0.37	7.34 ± 0.38
3.	*Apodachlya pyrifera*	0	13.76 ± 0.42	10. 09 ± 0.42	7.06 ± 3.25	5.65 ± 0.31
4.	*Botryodiplodia theobromae*	11.00 ± 0.10	13.76 ± 0.42	0	0	10.17 ± 0.48
5.	*Bovularia sp.*	0	0	9.17 ± 0.36	7.65 ± 0.57	0
6.	*Ceratocystis fimbriata*	0	11.93 ± 1.06	0	0	9.04 ± 0.43
7.	*Cercospora appi*	6.37 ± 0.39	0	14.29 ± 0.49	7.06 ± 0.32	0
8.	*Chalaropsis sp.*	0	0	9.17 ± 0.36	5.88 ± 0.28	5.65 ± 0.31
9.	*Dacromyces deliquescens*	6.37 ± 0.39	11.93 ± 1.06	0	0	7.34 ± 0.38
10.	*Fusarium oxysporum*	7.64 ± 0.42	12. 84 ± 0.32	0	0	10.17± 0.48
11.	*Geotrichum sp.*	6.37 ± 0.39	0	13.19 ± 0.46	8.82 ± 0.42	9.60 ± 0.45
12.	*Mucor mucedo*	8.25 ± 0.41	0	0	8.24 ± 0.39	10.17 ± 0.48
13.	*Penicillium vermiculatum*	5.73 ± 0.21	0	0	7.65 ± 0.37	0
14.	*Penicillium thomii*	5.73 ± 0.17	0	0	8.26 ± 0.39	0
15.	*Phoma herbarum*	0	8.26 ± 0.26	9.17 ± 0.26	0	5.65 ± 0.31
16.	*Rhizopus stolonifer*	8.28 ± 0.41	0	14.29 ± 0.49	8.82 ± 0.42	7.34 ± 0.38
17.	*Thielaviopsis brasicola*	9.55 ± 0.39	0	0	0	6.78 ± 0.36
18.	*Trichoderma viride*	0	0	0	8.24 ± 0.39	7.34 ± 0.36
19.	*Trichosporonoide oedecephalos*	0	9.17 ± 0.42	0	0	0
20.	*Graphium penicilliodes*	6.37 ± 0.39	13.76 ± 0.42	0	5.88 ± 0.28	7.90 ± 0.37

Note: values are means of four replicates ± standard error, 0 = No occurrence.

d) Soil analysis

Soil analysis revealed the presence of reasonable level of sand (24.5%), silt (60.5%) and clay (20.8%) as well as macronutrients potassium (K) 139% and phosphorus (P) 63%, magnesium (Mg) 145% and calcium (Ca) 109%, but low in Nitrogen (N) 20% and organic carbon (C) 1.89%. The soil P^H was 6.7% as presented in (Table 5).

Table 4 : Soil analysis

Soil constituents	% content
Texture (%)	
Sand	24.5
Silt	60.5
Clay	20.8
P^H	6.7
Nutrients (mg/kg)	
Nitrogen (N)	20
Organic carbon (C)	1.89
Phosphorus (P)	63
Potassium (K)	139
Magnesium (Mg)	145
Calcium (Ca)	109

e) Koch's postulates and pathogenicity tests

Pathogenicity (degree of infection) of fungi isolated from different parts of *G. arborea* and soil and their mean percentage infection is presented in (Table 5).

Results from the study showed that *Fusarium oxysporum*, *Botryodiplodia theobromae* and *Thievaliopsis brasicola* showed significant pathogenicity of 95 ± 2.5%, 75 ± 2.28% and 75 ± 2.21% respectively (at $p < 0.05$) on the root of *G. arborea*. *Fusarium oxysporum* was also pathogenic on the bark (stem) but to a lesser extent (48 ± 1.15%), *B. theobromae* was also pathogenic on the bark (47 ± 1.25%) while the effect of *Thievaliopsis brasicola* was not detected on any other part except the root. Of all the ten fungi isolated from the bark of *G. arborea*, the three most pathogenic ones were *Apodachlya pyrifera* (75 ± 2.25%), *Ceratocystis fimbriata* (65 ± 2.25%) and *Dacromyces deliquecens* (50 ± 1.78%). *Apodachlya pyrifera* was also pathogenic to the leaf and fruits, 48 ± 1.14% and 28 ± 0.98% respectively. Except the root, *Ceratocystis fimbriata* also affected the leaf (49 ±2.9%) and fruits (39 ± 1.02%), while *Dacromyces deliquescens*, also affected the root (26 ± 0.61%). Out of the nine fungi isolated from the leaf of G. arborea, the three most pathogenic ones were *Cercospora apii* (65 ± 2.07%), *Bouvularia* and *Chalaropsis* species with percentage infection of 50 ± 0.65% each. *Cercospora appi* was also pathogenic to the fruit (46 ± 0.88%) and root (27 ± 0.16%). *Bouvularia sp* was also pathogenic to fruit (46 ± 0.87%) while *Chalaropsis sp* was slightly pathogenic to the fruit (29 ± 0.31%) at $p < 0.05$. The three most pathogenic fungi out of the fourteen isolated from *G. arborea* fruit were *Trichoderma viride* (70 ± 2.31%), *Geotrichum sp* (50 ± 0.23%) and *Graphium penicilloides* (50± 0.23%). *Geotrichum sp* affected other parts except the stem, while *Graphium penicilloides* was also pathogenic to the root (43 ± 0.28%) and the stem (47 ± 0.34%).

Table 5 : Degree of infection (pathogenicity) by fungi isolated from various parts of *G. arborea* Roxb

S/N	Fungi	Percentage (%) infection of parts			
		Root	Bark	Leaf	Fruit
1.	*Aspergillus flavus*	43 ± 10.38	36 ± 0.96	21 ± 0.50	48 ± 1.03
2.	*Aspergillus niger*	38 ±0.99	41 ± 1.02	0	36 ± 0.82
3.	*Apodachlya pyrifera*	0	75 ± 0.05	48 ± 1.14	28 ± 0.98
4.	*Botryodiplodia theobromae*	75 ± 2.38	47 ± 1.25	0	0
5.	*Bovularia sp.*	0	0	50 ± 0.65	46 ± 0.87
6.	*Ceratocystis fimbriata*	0	65 ± 2.25	49 ± 2.09	39 ± 1.02
7.	*Cercospora appi*	27 ± 0.16	0	65 ± 2.07	46 ± 0.88
8.	*Chalaropsis sp.*	0	0	50 ± 0.65	29 ± 0.31
9.	*Dacromyces deliquescens*	26 ± 0.61	50 ± 1.78	0	0
10.	*Fusarium oxysporum*	95 ± 2.54	48 ± 1.15	0	0
11.	*Geotrichum sp.*	60 ± 0.20	0	39 ± 1.01	50 ± 0.21
12.	*Mucor mucedo*	31 ± 0.39	0	0	27 ± 0.41
13.	*Penicillium vermiculatum*	28 ± 1.36	0	0	38 ± 1.00
14.	*Penicillium thomii*	28 ± 0.20	0	0	42 ± 0.00
15.	*Phoma herbarum*	0	48 ± 1.17	48 ± 1.09	0
16.	*Rhizopus stolonifer*	38 ± 1.01	0	32 ± 0.76	44 ± 1.06
17.	*Thielaviopsis brasicola*	75 ± 2.24	0	0	0
18.	*Trichoderma viride*	0	0	0	70 ± 2.31
19.	*Trichosporonoide oedecephalos*	0	41 ± 1.00	0	0
20.	*Graphium penicilliodes*	43 ± 0.28	47 ± 0.34	0	50 ± 0.23
	CONTROL	-	-	-	-

The affected root and stem showed a marked reduction in dry weight when compared with the control, the lengths of the stem were shorter in the affected ones, and there were also dead spots on the stems. In the case of the leaf, the numbers of leaf spot depended on the fungus applied, and this varied significantly. The pathogenicity of the different fungi on the fruit showed significant differences based on the fungus applied and the rate of deterioration.

IV. Discussion

The wood of *Gmelina arborea* Roxb is suitable for general utility purposes especially in construction and structural work, carpentry, packaging utility, furniture, decorative veneers, light flooring, musical instruments, particle boards, electric poles, sawn timbers, valuable source of timber, pulp and fodder. This species has been extensively used in afforestation programmes, and has wider involvement in folk medicine. In spite of the economic potential of this tree in Nigeria, very little pathological research had been done on diseases afflicting this valuable species. This work involved extensive investigation of the fungal pathogens that affect *G. arborea* Roxb and the diseases they cause. In this study, twenty pathogenic fungi were isolated from various parts of *G. arborea* including roots, bark, leaf, fruit and rhizosphere (soil). The fungi were: *Aspergillus flavus* isolated from infected root, bark, leaf and seed of *G. arborea* while *A. niger* was isolated from infected root, bark, seed and soil. *Apodachlya pyrifera* was isolated from infected (bark, leaf, seed and soil), *Botryodiplodia theobromae* (root, bark and soil), *Bouvularia sp* (leaf and soil), *Ceratocystis fimbriata* (bark and soil), *Cercospora appi* (root, leaf and seed), *Chalaropsis sp* (leaf, seed and soil), *Dacromyces deliquescens* (root, bark and soil), *Geotrichum sp* (root, leaf, seed and soil), *Mucor mucedo* (root and seed), *Fusarium oxysporum* (root, bark and soil), *Penicillium vermiculatum* (root and seed), *Penicillium thomii* (root, seed and soil), *Phoma herbarum* (bark and leaf), *Rhizopus stolonifer* (root, leaf, seed and soil), *Thielaviopsis brasicola* (root and soil), *Trichoderma viride* (seed and soil), *Trichosporonoide oedocephalos* (bark) and *Graphium penicilloides* (root, bark, seed and soil). In this study, *Aspergillus flavus, Aspergillus niger, Cercospora appi, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Sclerotium rolfsii, Thielaviopsis brasicola and Graphium penicilloides* that were isolated from the root of *Gmelina arborea* agreed with the report of Inyang (1990). Tsao (1983) observed that some of these isolated fungi were isolated from the root of rubber plant (*Hevea brasiliensis*) and were pathogenic to the plant. The following fungi *A. flavus, A. niger, Apodachlya pyrifera, B. theobromae, Ceratocystis fimbriata*, *Dacromyces deliquecens, Fusarium oxysporum, Phoma herbarum* and *Trichosporonoide oedecephalos* isolated from the bark of *G. arborea* tree and found to be highly pathogenic to the plant especially at the seedling stage agrees with the findings of Inyang (1990). *A. flavus*, *Apodachlya pyrifera, Bouvularia sp, Cercospora apii, Geotrichum sp, Phoma herbarum* and *Rhizopus stolonifer* isolated from the leaf of *G. arborea* agrees with the findings of Duke (2002). Of the twenty fungi isolated, only four were not found in the soil (rhizosphere surrounding the tree). This finding is in conformity with the observations of Inyang (1990). In the fruit of *G. arborea* only *Botryodiplodia theobromae*, *Ceratocystis fimbriata*, *Dacromyces deliguecens* and *Thielaviopsis brasicola* were amongst the twenty isolated fungi. This finding agrees with the work of Duke (2002) who reported on the pathogenicity of *C. fimbriata*, *B. theobromae* and *T. brasicola* on the seed of *Gmelina arborea*.

In this study, some major fungal diseases were observed in the field during the survey. The diseases were leaf spot, stem canker, die bark, butt and root rot and damping-off. These findings are in conformity with the works of Orwa *et al.,* (2009), Nair and Sumardi (2000) who reported wilting in 1-2 month old seedlings, damping-off disease, which caused high seedling mortality, root-collar disease on 4-month-old seedlings and Anthracnose disease in nurseries. Plantation diseases observed by these researchers included leaf spot, vascular necrosis and chlorosis, heart rot and root rot, stem and branch canker (machete disease) and a bark disease (worm disease) that can girdle the base of the tree and cause die- back of branches in 2-year-old plantations.

In this study, we observed that all the twenty isolated fungal pathogens were pathogenic on *G. arborea*. The affected root and stem showed a marked reduction in dry weight when compared with the control, the lengths of the stem were shorter in the affected ones, and there were also dead spots on the stems. In the case of the leaf, the numbers of leaf spot depended on the fungus applied, and this varied significantly. The pathogenicity of the different fungi on the fruit showed significant differences based on the fungus applied and the rate of deterioration. These findings agree with that of Duke (2002), Inyang (1990) and Taysum (1987) who reported similar observations on different parts of *G. arborea* inoculated with the different isolated fungal pathogens.

V. Conclusion

Pathogenic fungi and fungal diseases associated with *G. arborea* in different locations in Cross River State, Nigeria were investigated. Twenty pathogenic fungi (*Aspergillus flavus, Aspergillus niger, Apodachlya pyrifera, Botroyodiplodia theobromae, Bouvularia sp, Ceratocystis fimbriata, Cercospora appi,*

Chalaropsis sp, Dacromyces deliquescens, Fusarium oxysporum, Geotrichum sp, Mucor mucedo, Penicillium vermiculatum, Penicillium thomii, Phoma herbarum, Rhizopus stolonifer, Thielaviopsis brasicola, Trichoderma viride, Trichosporonoide oedocephalus and *Graphium penicilliodes*) were isolated from bark, root, leaves, fruit and rhizosphere while a total number of five fungal diseases (leaf spot, stem canker, die bark, butt and root rot and damping-off) were identified (observed) in the field. Of the twenty fungal isolates, A. *flavus* occurred in all parts of the plant and the soil while others occurred in four or three parts. Pathogenicity tests revealed that all the isolated fungi were pathogenic on *G. arborea*. Due to the major role played by this plant in the economy of Cross River State, Nigeria, and the menace caused by the isolated fungal pathogens on *G. arborea,* further research will be carried out by these authors on the use of plant extracts in controlling these fungi.

VI. Acknowledgement

We express our profound gratitude to the management and staffs of the Cross River State Forestry Commission for granting us permission to carry out this survey in the various *Gmelina arborea* plantations. We thank also the technical staffs of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria for the invaluable assistance during the practical aspect of the research work.

References Références Referencias

1. Ademiluyi, E. and Okeke, R. E. (1973). Studies on the specific gravity and fibre characteristics of *Gmelina arborea* in some Nigerian Plantations. Nigerian Journal of Sciences 13: 132 – 242.
2. Agus, C. S., Kita, H. K., Toda, H., Karyants, O. and Hardiwinita, S. (2002). Nutrient circulation characteristics with different site index for fast growing species of *Gmelina arborea* Roxb. Plantation forest in tropical region. Phytopathology, 91 (7): 1111 – 1119.
3. Anonymous. (2002). Gmelina: *Gmelina arborea* Roxb. ASEAN Regional Centre for Biodiversity Conservation. ASEAN Environmental Education Plan 2000 – 2005.
4. Barnett, H. L., Hunter, B. B., 1998. Illustrated genera of imperfect fungi 4th edition, APS press, St. Paul Minnesota, p. 32
5. Chijoke, E. O. (1986). Soil factors and growth of *Gmelina arborea* in Omo Forest Reserve. Forest Ecology and Management 23: 245 – 251.
6. Cromer, R. N., Kriedemann, P. E., Sands, P. I. and Stewart, L. G. (1993). Leaf growth and photosynthetic response to nitrogen and phosphorus in seedling trees of *Gmelina arborea*. Plant Physiology, 20: 83 – 98.
7. Dugan, F. M., 2006. The identification of fungi. APS press, St Paul Minnesota. p. 50
8. Duke, J. A. (2002). *Gmelina arborea* Roxb. Purdue University, Centre for New Crops and Plant Products. http:/www.hot.purdue.edu/newcrop/Duke energy/*Gmelina arbrea*.html. Retrival Date: 3/10/2002.
9. Ebele, M. I. (2011). Evaluation of aqueous plant extracts to control rot fungi in paw paw. Journal of Applied Biosciences 37:2419-2424.
10. Enemuoh, P. O. (1970). Development of Forest Resources in the Economic Advancement of Nigeria. In Proceeding of the Inaugural Conference of Forestry Association of Nigeria. October 22 – 28.
11. FAO (1989). Le *Gmelina arborea*. Bureau regionalde la FAO pours l'Afrique, Accra, Ghana.
12. Greaves, A. (1973). Site studies and associated productivity of Gmelina arborea in Nigeria. M. Sc. Dissertation, Department of Biological Sciences, University College of North Wales, Bangor.
13. Halverson, I. J., Clayton, M. K. and Handelsman, J. O. (1993). Population biology of *Bacillus cereus* UW 85 in the rhizosphere of field-grown soy beans. Soil Biological Biochemistry 25 (4): 485 – 493.
14. Haygreen, J. G. and Bonyer, J. L. (1982). Forests products and wood science: An introduction. Iowa. The State University Press. Pp. 20 – 30.
15. Inyang, L. E. D. (1990). Fungal butt and root diseases: threats to bioconservation of Nigeria forest. Nigerian Journal of Science Bioconservation 1: 98 – 115.
16. Julian, E. (1982). Plantation Forestry in the tropics. New York, Oxford University Press. P. 50.
17. Murphy, J. and Riley, J. P. (1972). A modified single selection method for determination of phosphate in natural water. Analytical Chemistry Acta 27: 31 – 36.
18. Nair, K. S. S. and Sumardi, R. (2000). Insect pests and diseases of major plantation species. In Nair, KSS (ed.).Insect pests and diseases in Indonesia forests. CIFOR, Indonesia. Pp 15 – 37.
19. Ogbonnaya,C. I. M., Nwalozie, C. and Nwaigbo, L. C. (1992). Growth wood properties of *Gmelina arborea* (Verbanaceae) seedlings grown under five soil moisture regimes.American Journal of Tropical Botany, 79(2): 128 – 132.
20. Orwa,C. A. M., Kindt, R. and Jamnadass, R. S. A. (2009). Agroforestry Database: a tree reference and selection guide version 4.0. Http//www. Worldagrofestry.org/sites.
21. Richter, H. G. and Dallwitz, M. J. (2000). Commercial Timbers: Descriptions, illustrations, identification and information. Retrieval: English Version 4th May, 2002. http:/biodiversity.uno.edu/delta.
22. Tsao, P. H. (1983). Factors affecting isolation and quantification of Phytophthora from soil: Phytophthora, its biology, taxonomy and pathology (Eds Ewim, D. C., Barthnicki-gracia, S. and Tsao, P. H., St Paul American Phytopathological Society.

23. Yeoman's, J. C. and Bremner, J. M. (1989). A rapid and precise method for routine determination of organic carbon in soil. Community Soil Science and Plant Analysis 19: 1467 – 1476.