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Damping-off Disease of two Pulp and Paper Forest Species (*Pinus caribaea* Morelet and *Pinus oocarpa* Schiede) in the Nursery (Pp. 275-282)

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Abstract
An experiment was conducted to assess the effect of four sowing media (ground granite, sharp river sand, top soil and saw dust) on the incidence of damping-off disease in two pine species, Pinus caribaea and Pinus oocarpa during the wet and dry seasons of 2006/07. The experiment was laid out in a completely randomised design (CRD) replicated three times. A significant effect (P<0.05) was observed as top soil recorded the highest disease incidence in both species. There was no significant effect (P>0.05) between the ground granite and sharp river sand. Saw dust had 0% disease incidence and supported the highest plant height. Fusarium oxysporum was implicated as the causal agent of the disease. There was an inter-seasonal variation in

the disease occurrence in the study area as it was more severe in the rainy season.

Key words: Fusarium oxysporum, Pinus caribaea, Pinus oocarpa, sowing media, disease incidence.

Introduction

Pinus caribaea (Morelet) and *Pinus oocarpa* (Schiede) belong to the family Pinaceae. The species were introduced to Nigeria as exotics. The trees can attain a height of 30m. The leaves are needle-like, evergreen and arranged in groups of 2 to 5. The ovules and seeds which develop from them are borne on the upper surface of scales which form cones (Keay, 1989). Pines are important pulp and paper forest species with good long fibre quality. They could also be used as timber.

A number of forest companies have committed themselves to intensive, long-term programs of pine plantation management including tree improvement and nursery production, (Salerno *at al*., 2003). Numerous diseases attack these species in forest nurseries. Damping-off is the most common disease that affects the seeds, germinant and young seedlings, (Huang and Kuhlman & Pandey *et al.,* 1990). The disease is caused mainly by soil borne pathogenic fungi among which *Fusarium* species have been considered particularly damaging (Bloomberg, 1981 and Lori *et al.,* 1999). These organisms invade young seedlings leading to heavy losses and general physiological alternations of the seedlings, resulting in root decay and rotten stems at the soil line. Affected plants show water soaking at ground level causing the seedlings to fall over and die.

According to Jones and Averre (2000), species of *Rhizoctonia, Fusarium, Sclerotium rolfsii and Macrophomina phaseolina* cause damping-off under warmer and drier conditions and these have seriously limited the large scale production of these plants in the area. Many workers (Bakshi, *et al.,* 1972; Maramorosch, *et al*., 1982 and Rehill and Sen-Sarma, 1982) have also reported serious diseases of forest species both in the nursery and in the field.

Several nurseries have experienced problems with damping-off and root rot diseases. Some of these disease problems can result from infected seeds and/or the sowing media (Lori *et al*., 1999). In order to gain insight into the

problems affecting pine nursery production in Port Harcourt area, the study was aimed at:

(1) identifying the fungal pathogen(s) responsible for the damping-off disease,

(2) determining the best sowing medium or media for raising pine species in the nursery.

Materials and Methods

The study was conducted at the nursery site of the Department of Forestry and Wildlife Management, University of Port Harcourt, Port Harcourt, Nigeria. Port Harcourt is located on latitude 4^0 38'N and longitude 6^0 31'E. The rainfall pattern is bimodal with peaks in June and September. Relative humidity is about 90% at optimum level, while average temperature range is between 25^0C to 35^0C.

The seeds used for the investigation were imported from the United States of America and had 98-99% viability. The seeds were surface disinfected in 0.5% sodium hypochlorite solution, rinsed in several changes of sterile distilled water before use.

The experiment was conducted during the wet and dry season of the 2006/07 cropping season. Four sowing media namely; ground granite, sharp river sand, top soils (rich in organic matter) and saw dust were employed in the investigation. The experiment had three replicates in a completely randomised design (CRD). Twelve germination trays were each filled with each of the four media namely ground granite, sharp river sand, top soils and saw dust, for each of the species. One hundred seeds were broadcast per tray, giving a total of 1,200 seeds per species. The trays were watered regularly under high humidity propagators.

Germination counts were done four weeks after sowing. Disease development was assessed eight weeks after seedling emergence, when the secondary leaves (needles) were formed. This was done by assessing the number of collapsed seedlings. Vertical sections of collapsed seedlings were made using flame sterilized scalpels and examined using hand lens. Percentage disease incidence was calculated using the formula below:

$$\% \text{ incidence} = \frac{\text{No. of collapsed seedlings}}{\text{Total no. of germinated seedlings}} \times 100 \quad (1)$$

The heights of the plants were also assessed 12 weeks after seedling emergence. Data was subjected to one-way analysis of variance (ANOVA) and significant differences among means were determined using least significant differences.

Infected plant materials brought back from the nursery were washed, cut into 5mm segments including margin of infection. 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 28^0C for 7 days during which the fungi growing out of the sections were isolated. The isolates were purified; pathogenicity tests and Koch's postulates were carried out.

Results and Discussion

Fusarium oxysporum was isolated in all cases. No slimy viscous drops were observed when stem cuttings were placed in water. This excluded *Pseudomonas sp* as the causative organism of the condition (Bailey, 1966 and Osuinde and Ikediugwu, 1996). The pathogenicity test confirmed *Fusarium oxysporum* as responsible for the damping-off disease of the species under investigation. Extensive damage was observed to be caused on the roots. This is the first report of *Fusarium* associated with damping-off of pine species in the area.

Table 1 shows % disease incidence in *Pinus caribaea* and *Pinus oocarpa* during the wet and dry seasons. In the wet season, top soil showed the highest disease incidence in both *Pinus caribaea* (38.24%) and *Pinus oocarpa* (45.67%) and their effects was significantly different ($P<0.05$) from the other sowing media (Table 1). Saw dust medium had 0% incidence. The effect of ground granite and sharp river sand were not significantly different ($P>0.05$) from each other in both species. The % disease incidence followed the same trend in the dry season except that it

Data are average of three replicates was higher in the wet season and *Pinus oocarpa* was more susceptible. The highest disease incidence observed during the wet season could be attributed to the high relative humidity (51-90%), temperature (20-33^0C) and high rainfall averaging 2,100mm annually associated with the season in the zone (Agromet Division NRCRI, 2006)

which favours diseases caused by fungi. *Fusarium* damping-off is an economically important disease of many Nigerian crops. This investigation has shown that *Fusarium* damping-off is a disease of these forest species at the seedling stage and this is in agreement with the works of James, (1999) and Lori and Salerno, (2002). This finding also gives credence the observation of Swaine *et al*., (1997), that successful regeneration of tropical forest plants from seeds to a dominant tree is influenced by several complex factors including biotic factors. These soil-inhabiting facultative parasites survive either as dormant resting spores or as active saprophytes on decaying organic matter in the top soil and act as the primary inoculum to incite disease wherever they come in contact with a susceptible host. Pandey *et al*., (1990) had reported *Fusarium spp* associated with ungerminated seeds of *Pinus roxburgii*. They reduce germination and cause pre-emergence damping-off as the *Fusarium*-contaminated seed produce weak germinant that die before the radicle emerges. Damage to these exotic species in the present study, may have been due to the sowing media used in the propagation as most of the seedlings emerged from the media before damping-off.

Table 2 shows that seedlings that escaped attack moderately did well as evident in the plant height. They were able to compete favourably with the pathogen for the available nutrients. The significant effect ($P<0.05$) of saw dust medium in both species may be attributed to the biodegradation of the saw dust by the organism which may have released the necessary nutrients needed for the plant growth or the attack on the saw dust may have exempted the seedlings from attack. There may have been limited absorbable nutrients for the growth of the seedlings in ground granite and sharp river sand media as evident in their plant height. Plant pathogens constitute serious impediments to the growth of seedlings in forest nurseries (Ogboghodo, 1998). They reduce the survival rate and cause serious propagation failures of the seedlings. Pine growers must be especially alert to prevent disease problems that can develop from sowing media and may spread quickly during early seedling development where succulent germinant may be killed within a few days.

Conclusion

Fusarium species are the primary source of inoculum and they interfere with the quality of planted seeds and the ability of crops to become established and realise their full potential of yield and value. It could therefore be

recommended that for most effective protection of young pine seedlings against *Fusarium* attack in the nursery, saw dust medium may be used in propagation. Integrated disease management approach may include the sterilization of top soil, ground granite, river sand or any nursery medium before use. Certain precautions during seed collection and handling practices should be taken to reduce the possibility of introducing seed-borne pathogens into the nursery as seeds contaminated with *Fusarium* species may build up *Fusarium* counts. Since the pathogen showed inter-seasonal variation in attack, propagation should be done mainly in the dry season.

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Table 1: Percentage incidence of damping-off disease in *Pinus caribeae* and *Pinus oocarpa* in four sowing media at the wet and dry seasons

Sowing Medium	Disease incidence (%)			
	P. caribaea		*P. oocarpa*	
	Wet season	Dry season	Wet season	Dry season
Ground granite	8.26	5.74	26.41	19.34
Sharp river sand	8.32	6.00	25.54	16.38
Top soil	38.24	18.39	45.67	30.76
Saw dust	0.00	0.00	0.00	0.00
LSD (0.05)	5.94	2.33	8.50	4.85

Table 2: Mean height (cm) of *P. caribaea* and *P. oocarpa* at 12 wks after seedling emergence in 4 sowing media at the wet and dry season

Sowing Medium	**Plant height (cm)**			
	P. caribaea		***P. oocarpa***	
	Wet season	**Dry season**	**Wet season**	**Dry season**
Ground granite	6.94	6.88	6.23	6.70
Sharp river sand	11.24	10.21	11.38	11.50
Top soil	11.86	11.78	13.61	12.64
Saw dust	15.66	14.32	15.68	15.42
LSD (0.05)	2.92	2.88	2.77	2.85

Data are average of three replicates.