

## Dry season and diurnal surveys of phylloplane fungi of *Hevea brasiliensis* in Nigeria

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Okhuoya J. A., Ahweyevu C. O.: Dry season and diurnal surveys of phylloplane fungi of *Hevea brasiliensis* in Nigeria. Acta Mycol. 23(1): 143-147, 1987 (1988).

Monthly and diurnal variation of phylloplane fungi of rubber (*Hevea brasiliensis*) leaves were studied over a period of four months in the dry season, using two culturing methods. Composite fungal population was the highest in April and the lowest in February. Serial dilution method recorded the higher number of fungal spores than ballistospore method. Mature leaves were found to have more fungal spores than premature and young leaves. Spore concentration on the leaves showed diurnal periodicity, with peak period of spores between 12-18 hr.

Rubber leaves outside the plantation, had more spores on their surfaces than those shaded by the plantation canopy. The factors responsible for these observations were discussed.

### INTRODUCTION

The phylloplane as a fungi habitat has been well documented in recent years (Last, Deighton 1965; Lebn 1965; Preece, Dickinson 1971; Pandey, Dwivedi 1984). Microorganisms inhabit leaves once they open up (Hogg and Hudson 1966; Roscoe 1971) and also persist even after leaf fall.

Rubber *Hevea brasiliensis* cultivated in Nigeria for latex production, is a very important economic plant. It is the fourth most important agricultural export product in Nigeria (Rubber Statist. Bull. 1983). In the Rubber Research Institute of Nigeria, two principal leaf pathogens have been found namely *Drechslera hevea* (causal organism for birds eye leaf spot) and *Colletotrichum gloeosporioides* (causal organism for gloeosporin leaf spot). Previous studies (Okhuoya 1985) have shown that these pathogens are endemic with peak

periods of occurrence in the air in the beginning of dry season. In this study, fungi colonising the rubber leaves, both under the plantation canopy and in the open (i.e. without canopy shade) were isolated. Their diurnal variation on the leaves was also determined.

#### MATERIALS AND METHOD

Leaves of rubber were collected from a rubber plantation in Ekosodin village near the campus of the University of Benin. Leaves of rubber seedlings under plantation canopy were regarded as those under shade while leaves obtained from seedlings outside the plantation were regarded as exposed leaves. These leaves were carefully excised from plants, put in sterile polythene bags and taken to the laboratory for study.

Mature dark green leaves from both shaded and exposed plants were sampled once a week using both serial dilution and ballistospore methods, from January to April.

In diurnal sampling, three categories of leaves were sampled at 6, 12 and 18 hours using both methods of sampling. The leaves were namely: young expanded brown leaves in the uppermost region of the stem, premature light green leaves in the middle region of the stem, and mature dark green leaves in the lowest region of the stem. Diurnal sampling was done once every month for four months using mature leaves under both canopy and outside the plantation. Fungi isolated were identified at least to the genus level using appropriate taxonomic keys.

Pathogenicity test was determined for all isolates through normal surface spraying of leaves (previously surface sterilized with 1% HgCl and sterile distilled water) with their spore and mycelial inocula. Also in another test, leaves were wounded slightly with abrasive paper before spraying with the inoculum. The development of lesions or leaf spots indicated positive test.

#### RESULTS

Fungal populations on leaves from both exposed and shaded plants varied from January to April. Exposed leaves had more fungal populations than shaded leaves (Table 1). Nineteen fungal species isolated, were mainly members of the *Deuteromycetes*. Mature leaves had more fungal spores per unit area than young expanded brown leaves. In diurnal sampling both exposed and shaded leaves had maximum fungal spores on their surface at noon.

Dark green leaves in the lowest region, had more fungal spores than young apical leaves. In general, the fungal population on leaves increased with the leaf age.

Table 1  
Fungi taxa isolated from phylloplane of rubber  
using serial dilution /A/ and balltistospore /B/  
methods

Taxa	A		B	
	exposed	shaded	exposed	shaded
<i>Alternaria</i>	+	+	-	-
<i>Aspergillus flavus</i>	+	+	+	-
<i>A. fumigatus</i>	+	+	-	+
<i>A. niger</i>	+	+	+	+
<i>Aureobasidium pullulans</i>	+	+	+	-
<i>Botrytis</i>	+	-	-	-
<i>Cladosporium</i>	+	+	-	-
<i>Epicoccum</i>	-	-	-	+
<i>Geotrichum</i>	+	+	+	-
<i>Drechslera</i>	+	-	+	-
<i>Neurospora</i>	+	+	-	-
<i>Penicillium</i>	+	+	-	-
<i>Rhizopus</i>	-	+	-	-
<i>Rhodotorula</i>	-	-	+	-
<i>Sporobolomyces</i>	+	+	+	+

Some fungi were permanently isolated while others appearance varied (Table 1), both on shaded and exposed leaves. *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Colletotrichum*, *Helminthosporium* were found to be pathogenic while the others were saprophytes. However, only *Colletotrichum* and *Helminthosporium* were able to develop leaf spots without wounding. This indicated that they were strong pathogens.

#### DISCUSSION

Fungal population decreases from January to February and then gradually increases till April. In Nigeria during this period rubber plants defoliate and refoilate. By January most rubber trees begin defoliation and complete in by March. In March, fresh flushes of leaves begin to appear and refoilation is completed by April. The pattern of fungal spore concentration during this period tends to correlate with the defoliation-refoliation of rubber leaves. Seasonal changes have been established to affect the phylloplane fungi of some plants especially in the tropics (Fell, Hunter 1979; Pandey, Dwivedi 1984).

The bigger fungal population on mature than on young leaves suggests that the former have more nutrients on their surface which attract more micro-organisms. Also the longer period of exposure to the atmosphere due to their age provides for more fungal colonisation (W e j d e, D e s p h a n d e 1979). This is further confirmed by the observation that dark green leaves in the lowest region of a plant have more spores than young apical leaves. Rubber leaves under the plantation canopy have less composite fungal spores than exposed ones. Similarly O k h u o y a (1985) has found that there are more fungal spores outside the plantation canopy than inside a plantation. Thus, the low fungal spore load in the air under a plantation may in part account for the small number of phylloplane fungal spores on leaves of shaded rubber seedlings.

In diurnal sampling, two categories of leaves have maximum spore concentration between 12-18 hrs. This period is often associated with high temperatures and sunshine during daytime in Nigeria, which aid spore liberation and dispersal from their substrates (G r e g o r y 1971). Leaf pathogens of rubber have been found to have their peak period in the air at midday (O k h u o y a 1985). The increased fungal concentration on leaves under shade in February could be due to the reduction of canopy shade as a result of leaf fall. This appears to support the view that the leaves that make up the canopy act as physical barrier in preventing spore deposition on the leaves of seedlings under them.

Serial dilution method has proved to be a more suitable technique for quantitative sampling than the ballistospore. This method has been recognised by other workers as a very effective technique for detecting phylloplane fungi (P a n d e y, D w i v e d i 1984; S i n g h et al. 1984). *Sporobolomyces roseus* has been consistently isolated from the leaves irrespective of their age. This observation agrees with that of R o s c o e (1971). Among the pathogens isolated, only *Cladosporium*, *Colletotrichum*, *Fusarium*, *Drechslera*, have been isolated from leaves of all ages, while others have only been found, on young leaves. Although more phylloplane fungi have been found to be pathogenic than the usual two pathogens commonly found here in Benin City, among them only *Drechslera* and *Colletotrichum* are strong pathogens. However, other weak pathogens become effective only when leaves are injured or bruised. Since leaf bruises are not common, except insect damage, diseases caused by these weak pathogens are rare in the Benin area. Epiphytic microbes on the leaf surface play an important role in the control of plant diseases. They produce growth suppressing substances (antibiotics) against pathogens or quickly use up the available energy resources on leaves, which might have been conducive for the rapid growth of pathogens (L a s t, D e i g h t o n 1965). Further studies are required to establish the phenomenon operating in the case of the rubber phylloplane.

We are grateful to the Head and technical staff of Botany Department of the University of Benin, Benin City, Nigeria, for their assistance and advice.

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