

## NEEDLE CAST OF PINUS ELLIOTTII AT FOREST RESEARCH INSTITUTE CAMPUS, CHITTAGONG

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Tropical pines have been under introduction trials in Bangladesh since 1961-62 with a view to building up a source of long-fibred pulp. Pathological observations in respect of planted Pines at Forest Research Institute Campus, Chittagong are reported. The etiology and symptoms of needle cast disease of *Pinus elliottii* Engelm. have been described.

*Botryodiplodia theobromae* Pat. and *Pestalotia macrotricha* Kl. were mainly isolated from the infected needles. Pathogenicity tests were carried out. The results indicated that both the fungi were responsible for the development of needle cast of *P. elliotti*, the former having a dominant role. The disease, however, was not a serious threat to the crop and has not been recorded on other species of Pines in the campus.

### INTRODUCTION

Trials on introduction of tropical Pines have been undertaken in Bangladesh with a view to building up a source of longfibred pulp. *Pinus caribaea*, *P. oocarpa* and *P. elliottii* have been experimentally planted over the last 15-20 years at the Bangladesh Forest Research Institute Campus.

Needle cost is a general term used to describe diseases in which needles are shed from diseased trees. Specific needle cast differs with respect to age of needles

attacked and time of year affected, depending on the species of fungus and the infected host. However, most needle casts have some common characteristics (Blanchard and Tattar 1981). They occur on Pines, Spruces, Firs, Larches and various Ceders. Defoliation is rarely severe enough to kill any trees except young seedlings (Baxter 1952, Boyce 1961). In some needle casts infection is mostly restricted to the newly developing needles, and sporulation is timed to coincide in spring, when new needles develop (Smith 1978). The disease

is mainly caused by certain genera of the Ascomycetes (Hypodermataceae) and the Fungi Imperfecti, by environmental factors or a combination of fungi and adverse conditions.

During 1977-78 an investigation was made to observe the pathological conditions of planted Pines at Forest Research Institute Campus. It was observed that some of the *P. elliotii* trees showed defoliation of current year's needles. The present paper deals with the study of the symptoms and causal organism of needle cast.

## MATERIALS AND METHODS

Affected needles and apparently healthy needles were collected from severely affected shoots of *P. elliotii* trees. One, two, and three year-old healthy and affected needles were collected at random from sixteen trees. Collected samples were compared with each other and the types of infection noted. Infected needles bearing mature spots, and apparently healthy needles of different ages were also selected for isolation of fungi.

The samples under last were washed in running tap water and then in dilute soap water to remove the adhering dirt and subsequently washed with several aliquots of distilled water. The needles were then surface sterilized using 0.1% mercuric chloride solution for 2 minutes (Booth 1971). The needles were finally treated with Streptomycin and Penicillin solution for 3 minutes (Anon. 1968). The samples were then cut aseptically into 1-2 mm small pieces. Infected and healthy needles were planted on 2% Malt Extract Agar (MA) and Potato Sucrose Agar (PSA) media (Anon. 1968). Plates were incubated at

25°C for 15 days and observed regularly. When sufficient mycelial growth became visible, the fungi were grouped visually, isolated and identified.

To find out the extent of incidence of infection on healthy and infected needles, about 500 needles of each of the types were collected at random from sixteen affected trees.

Newly flushed needles on shoots of sixteen *P. elliotii* trees were inoculated by spraying with mycelial suspension with a sterilized syringe and then each individual shoot was covered with a polyethylene bag to maintain high relative humidity for three days. Equal number of untreated shoots on all the trees were covered with polyethylene bag in the same as controls. Thereafter, observations were taken at weekly intervals.

## RESULTS

**Symptoms of the disease :** Generally the trees exhibited reduced growth and degenerated look. Two and three year old needles, mainly the latter, dropped in large numbers and were hanging amidst green needles. Such dead needles and a proportion of apparently healthy ones showed reddish-brown lesions on the needles within the sheath region. Besides such necrotic areas, scattered brown spots developed on the three year old needles.

Reduced current year's shoot growth was observed. Some of the shoots failed to flush while others flushed partially. When such needles were about 1-2 cm, necrotic lesions, as above, were found to occur on a small proportion of needles. Incidence of symptoms on the diseased needles was

significantly high ( $P=0.001$ ) as compared to that on the apparently healthy ones (Table 1). Examination of apparently dying shoots and roots of affected trees did not reveal either shoot dieback or root rot diseases.

**Isolation of fungi :** The results of isolation of fungi, as presented in Table 2, show that basically two fungi, identified as *Botryodiplodia theobromae* Pat. (Type-b) and *Pestalotia macrotricha* K1. (Type-a), were obtained. The former was dominant of the two.

growing mycelial culture of *B. theobromae* and *P. macrotricha*, presented in Table 2, revealed that both the fungi were able to infect uninjured needles significantly ( $P > 0.001$ ) as compared to the controls. Symptoms, as found to occur naturally, continued to develop from 7 to 35 days. It may, therefore, be mentioned that in case of an insignificantly small number of the controls slight infection symptoms developed by 35 days while significantly ( $P=0.001$ ) higher number of the inoculated branches developed severe to moderate levels of infection by 28 days (Table 3).

**Table 1. Incidence of disease symptoms on needles of *P. elliottii***

Needles Types (3 age classes)	No. of needles assessed	Needles infected (%)
Diseased	8000	63.25***
Healthy (apparently)	8000	36.75

\*\*\*Signifies  $P=0.001$

**Table 2. Isolation of fungi from infected and healthy young needles of *P. elliottii***

Age of needles	Needles Types	No. of needle pieces plated	Percent isolation	
			a	b
1	Infected	50	40	60
	Healthy	50	0	0
2	Infected	50	44	56
	Healthy	50	0	0
3	Infected	50	44	56
	Healthy	50	0	0

**Pathogenicity tests :** Results of artificial inoculation of young twigs of *P. elliottii* by 20 days old actively

It is evident from Table 3 that *B. theobromae* could be consistently reisolated from the infected needles. Reisolation of

Table 3. Extent of infection on artificial inoculation of *P. elliotii* and result of reisolation of fungi

Fungi	No. of trees	No. of branch inoculated	No. of branch with symptoms	Level of infection				Reisolation	
				+++ (severe)	++ (moderate)	+ (slight)	- Nil	No. of inocula plated	% isolation
<i>B. theobromae</i>	16	48	30	15	15	0	0	260	73***
<i>P. macrotricha</i>	16	48	30	12	12	6	0	260	28**
Control	16	48	12	0	0	12	36	130	4

\*\*\*=0.001, \*\*P=0.01

this fungus was significantly higher ( $P=0.001$ ) as compared to either of the controls or the *P. macrotricha* while the latter was also reisolated significantly ( $P=0.01$ ) as compared to the controls. This also confirms the dominant role of *B. theobromae*.

## DISCUSSION

Infection took place most frequently on one year old needles, but also occurred on 2 year old needles being most prevalent on 3 year old needles. Affected needles usually shed in the late spring but before defoliation was noticed in the early summer.

Review of literature indicates that *B. theobromae* was the first reported needle can fungus on *P. elliotii* tree in Bangladesh. This is a wide spread saprophyte and a facultative wound parasite in the tropics and subtropics causing diseases in living plants. It also causes preharvest and storage rots of fruits, dieback, canker, staining of timber and damping off. This fungus caused dieback of *Albizia*

*falcata* *A. sumatrana* and *A. julibrissin* in Indonesia, India, U. S. A. and Iran (D'Angremond 1948, Venkata Ram 1960, Browne 1968, Spaulding 1956, 1958, 1961, Scharif 1964). This also causes dieback of *Artocarpus integrifolia* (Kathal) in Bangladesh (Rahman *et al* 1984, Unpub. data) and *Hevea brasiliensis* in Sri Lanka (Murrey 1930). *B. theobromae* caused cankers on young *P. elliotii* and *P. taeda* in Australia (Young 1936, Browne 1968) and root rot of *P. caribaea* in Sabah, Malaysia (Liu 1977).

Even though *P. macrotricha* was isolated from infected needles and produced symptoms on the trees after inoculation could be less consistently reisolated as compared to *B. theobromae*. This fungus is often listed as a pathogen (Gibson 1975, 1979).

*B. theobromae* is transmitted by wind and water (Meredith 1961). So it was suspected that some of the controls became contaminated by air-borne or water transmitted spores of the fungus occurring naturally. Both the fungi were also reisolated

from the infected controls. Though *B. theobromae* played a dominant role as needle cast of *P. elliottii* tree at Forest Research Institute Campus, it did not pose any serious threat to other planted Pines there.

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