



***A PLANT PATHOGENIC FUNGUS ELICITING NECROSIS (LEAF SPOT)
AGAINST *Exbucklandia populnea* (R.Br. ex Griff.) R.W.Br. (GEROK) AT
FOREST NURSERY TERLA B***

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FOREST NURSERY TERLA B**



By

TATSURO KIKUCHI

**A Project Report Submitted in Partial Fulfilment of the Requirements
for the Degree of Bachelor of Forestry Science in the
Faculty of Forestry
Universiti Putra Malaysia**

2019

DEDICATION

Specially dedicated to my:

Parents

Dr. Razak Bin Terhem

Assoc. Prof. Dr. Mohd Nazre Saleh

Prof. Dr. Arata Momohara

Thank you for your encouragement and support

And all the sacrifices that you have given to me

Thank you for everything

ABSTRACT

Exbucklandia populnea is an important species for a restoration programme in Cameron Highlands as it is a common pioneer tree in Cameron Highlands. However, necrotic disease was observed and killed a lot of seedlings of *E. populnea* at Forest Nursery Terla B, which is the only nursery for the restoration in Cameron Highlands. Further spread of the disease is considered to be able to decrease the supply of seedlings of *E. populnea* because the germination rate of the seeds is low, and the seedlings grow slowly. Therefore, the purpose of this study is to determine the causal agent of the disease and estimate the impact in the nursery. From a survey at the nursery, nearly 30 % of *E. populnea* seedlings showed necrotic disease, which made it the most dominant disease in the nursery, and the necrotic symptom was categorized into two sub-symptoms: TS (small brown spots) on young leaves and TB (big brown spots) on both old and young leaves. The symptomatic leaves of *E. populnea* were collected at the nursery, and the necrotic tissues were cut into small pieces. The pieces and the spores on the spots were placed onto potato dextrose agar media and incubated at room temperature (27-30 °C). From both of TS and TB symptomatic leaves a grey colonial fungus named T1 was isolated. T1 had identical spores and hyphae that were confirmed on both TS and TB symptomatic leaves. Thus, T1 was suspected as the causal agent of both of the types of leaf spot diseases. All strains of T1 were identified as *Botrytis cinerea* by DNA sequencing of ITS region. The fungus is notorious for causing necrotic diseases on plants in nurseries. Mycelial suspension and mycelial plugs were used as inoculums for the pathogenic tests. During the tests, TB symptom was confirmed on the inoculated leaves, but TS symptom did not occur. Thereby, it was not fully confirmed in this study that TS symptom was caused by *B. cinerea*, although this symptom was highly likely caused by *B. cinerea*. TS symptom was considered to be not simply caused by surface inoculation and might involve physiological change of *B. cinerea* from endophytic to necrotrophic life style as previous research shows. Further research about the relationship between *E. populnea* and *B. cinerea* would help to find specific measures to control the necrotic diseases.

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APPROVAL SHEET

I certify that this research project report entitled "A Plant Pathogenic Fungi Eliciting Necrosis against *Exbucklandia Populnea* (R.Br. Ex Griff.) R.W.Br. (Gerok) at Forest Nursery Terla B" by Tatsuro Kikuchi has been examined and approved as a partial fulfilment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Univeristi Putra Malaysia.

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LIST OF ABBREVIATIONS

FDPM	Forest Department of Peninsular Malaysia
PRF	Permanent Reserved Forest
FNTB	Forest Nursery Terla B
PCR	Polymerase Chain Reaction
ITS	Internal Transcribed Spacer
NCBI	National Centre for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
UPM	Universiti Putra Malaysia
PDA	Potato Dextrose Agarose
RT	Room Temperature
RL	Room Light
UV	Ultraviolet
S	Svedberg
rDNA	ribosomal DNA
FRIM	Forest Research Institute Malaysia
Voltages	V
TS	Type Small (small brown spot)
TB	Type Big (big brown spot)
RGR	Radial Growth Rate
bp	Base Pairs

CHAPTER 1

INTRODUCTION

1.1 Background

Cameron Highlands is an extremely important area in the world in biological and ecological aspects of the cloud forest. The cloud forest is limited to high elevation areas and provides habitats of many endemic species that are adapted to high altitude environments (Peh *et al.*, 2011). However, the rich biodiversity of Cameron Highlands has been faced with a lot of human induced problems; unsustainable and unregulated farming, development of residential and resort areas and construction of unsustainable roads. Among them, farming is considered to be one of the major causes of the nature degradation in this area because farming process involves discharge of pesticides and fertilizers, and illegal forest encroachment to expand farmlands for lucrative highland crops. Expansion of farmlands has led to enormous destruction of habitats of the indigenous species and vegetation cover, which promotes landslides and soil erosion on the unstable slopes (Chan, 2006). These landslides have damaged the properties (Pradhan & Lee, 2009).

Following the catastrophic events, the Cabinet permitted for the Ministry of Water, Land and Natural Resources to implement series of restoration programmes for the degraded forests. In 2015 Forest Department of Peninsular Malaysia (FDPM) was established to carry out the program under the 11th Malaysia Plan with an allocation of RM100 million from 2017 to 2020. This program is planning plantation of 1,025,000 trees in permanent reserved

forest (PRF) areas in Peninsular Malaysia by 2020. In Cameron Highlands, FDPM is going to plant 312,500 trees within about 500 ha land of the PRF by 2020 (FDPM, 2018). As well as FDPM, public groups such as non-governmental organizations and local secondary school students help to plant 40,000 trees within about 40 ha land of the PRF in Cameron Highlands by 2020 (Mohd, 2018).

FDPM selected the following species for the restoration program in Cameron Highlands: *Hopea odorata* (Merawan Siput Jantan: Dipterocarpaceae); *Magnolia elegans* (Cempaka Hutan: Magnoliaceae); *Shorea platyclados* (Meranti Bukit: Dipterocarpaceae); *Casuarina* sp. (Ru: Casuarinaceae); *Parkia speciosa* (Petai: Fabaceae); *Neolamarckia cadamba* (Kelempayan: Rubiaceae); *Exbucklandia populnea* (Gerok: Hamamelidaceae) (Mohd, S. & Mohd, J., personal communication, October 22, 2018). In order to produce high quality seedlings, FDPM established a nursery named Forest Nursery Terla B (FNTB). However, necrotic disease with leaf spot, sub-circular brown lesion, was found on leaves of a lot of *E. populnea* seedlings at FNTB and led many of seedlings to death.

1.2 Problem Statement and Justification

E. populnea is an important species as a common tree for the restoration program in Cameron Highlands (Schmid *et al.*, 1998). However, the necrotic disease damages *E. populnea* seedlings in FNTB. Since FNTB is the only

forest nursery for the restoration in Cameron Highlands and seedlings of *E. populnea* grow very slowly and the germination rate of the seeds is low (Schmid *et al.*, 1998), further spread of the disease might lead to reduction of supply of *E. populnea* seedlings. Therefore, identification of the causal agent was needed to control the disease for management of the forest nursery.

Research on plant pathogenic fungi infecting *E. populnea* has been very limited since Bilgrami and Purohit (1970) described merely one plant pathogenic fungus: *Leptosphaeria* sp.. Thus, this study is expected to play an important role to make a new record of plant pathogenic fungi on *E. populnea*.

1.3 Objectives

- 1) To estimate the impact of necrosis (leaf spot) on *E. populnea* in FNTB
- 2) To isolate the causal agent of the necrosis (leaf spot) from *E. populnea*.
- 3) To identify isolates based on morphological and molecular analysis

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